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ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · APRIL 1990

Impact Factor: 4.72 · DOI: 10.1021/jo00296a034

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125567-70-6; **34**, 125567-78-4; **34** (diethyl acetal), 125567-58-0; (±)-**35**, 125567-79-5; (±)-**36** (isomer 1), 125567-80-8; (±)-**36** (isomer 2), 125638-48-4; (±)-**37** (isomer 1), 125567-81-9; (±)-**37** (isomer 2), 125638-49-5; (±)-**38b**, 125567-82-0; (±)-**39**, 125567-83-1; (±)-**39** (dihydro derivative), 59372-73-5; (E)-$(EtO)_2CHCMe_2CH_2CH{=}C(Me)COMe$, 125567-56-8; $(\pm)$-$(EtO)_2P(O)CHMeCOMe$, 117653-52-8; $Ph_3P{=}C(Me)CO_2Et$, 5717-37-3; cyclopentadiene, 542-92-7.

Synthesis of Mercapturic Acid Derivatives of Putative Toxic Metabolites of Bromobenzene[1]

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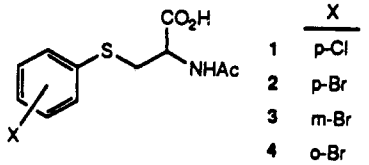
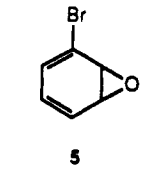
Received July 3, 1989

The synthesis and characterization of nine isomerically defined *S*-arylmercapturic acids of interest in connection with the metabolism of the model hepatotoxin bromobenzene is described. Included are three *S*-(bromophenyl)-, two *S*-(bromohydroxyphenyl)-, and three *S*-(bromodihydroxyphenyl)mercapturic acids of defined substitution pattern. In addition, several related compounds with two or no bromine atoms are described. These syntheses depend on two basic methods, 1,4-addition of various arene thiols to acetamidoacrylic acid or the 1,4-addition of *N*-acetyl-L-cysteine to various benzoquinone derivatives. In addition, we describe a method for efficient conversion of the mercapturic acids to thioanisole derivatives, regioisomers of which can be separated and detected at low levels by capillary gas-liquid chromatography.

Mercapturic acids are S-substituted derivatives of *N*-acetyl-L-cysteine (e.g., **1**-**4**). They are typically isolated from the urine of animals or humans exposed to electrophilic agents such as epoxides, enones, alkyl halides, etc.



	X
1	p-Cl
2	p-Br
3	m-Br
4	o-Br



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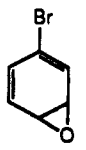

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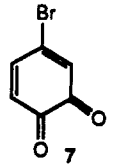

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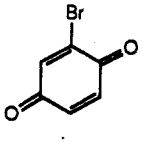

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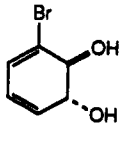

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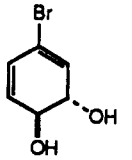

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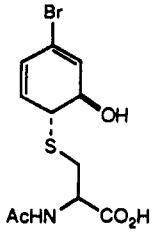

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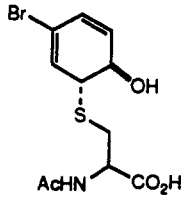

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Their biosynthesis involves reaction of the electrophile with the sulfhydryl group of glutathione (γ-Glu-Cys-Gly), usually with catalysis by one or more isozymes of glutathione transferase.[2] This is followed by enzymatic hydrolysis of the Glu-Cys and Cys-Gly bonds and N-acetylation.[3] *S*-(*p*-Chlorophenyl)- and *S*-(*p*-bromophenyl)mercapturic acids (**1** and **2**) were first isolated in 1879 from the urine of dogs treated with chloro- or bromobenzene.[4,5] Since then, mercapturic acid metabolites of a great many other drugs and chemicals have been isolated. Contemporary interest in mercapturic acids derives largely from the fact that their excretion implies the previous presence in the animal of an electrophile, many of which have been associated with toxic effects on living cells. With suitably sensitive analytical methods, urinary mercapturic acids can provide an approach to "molecular dosimetry" of workers exposed occupationally to electrophilic compounds or their metabolic precursors. For example, phenylmercapturic acid is found in the urine of workers exposed to benzene.[6]

Our current interest in mercapturic acids stems from a more general interest in the chemical basis for the hepatotoxicity of bromobenzene and certain derivatives of it.[7] Both in vivo and in isolated hepatocytes the toxicity of bromobenzene has been correlated with the covalent binding of one or more of its chemically reactive metabolites to cellular proteins; it is *presumed* that at least *some* of these covalent binding events are deleterious to the cell in which they occur. Both covalent binding and toxicity are enhanced by prior depletion of glutathione.

(1) Financial support for this research was provided by NIH Grant GM-21784. L.R.H. is a NIH Predoctoral Trainee (GM-07775).

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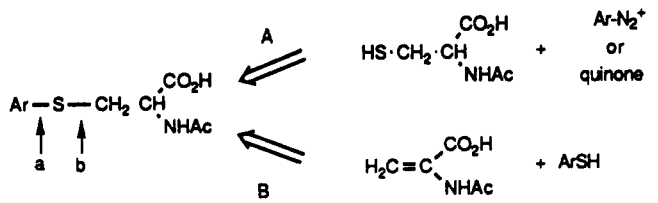
Scheme I



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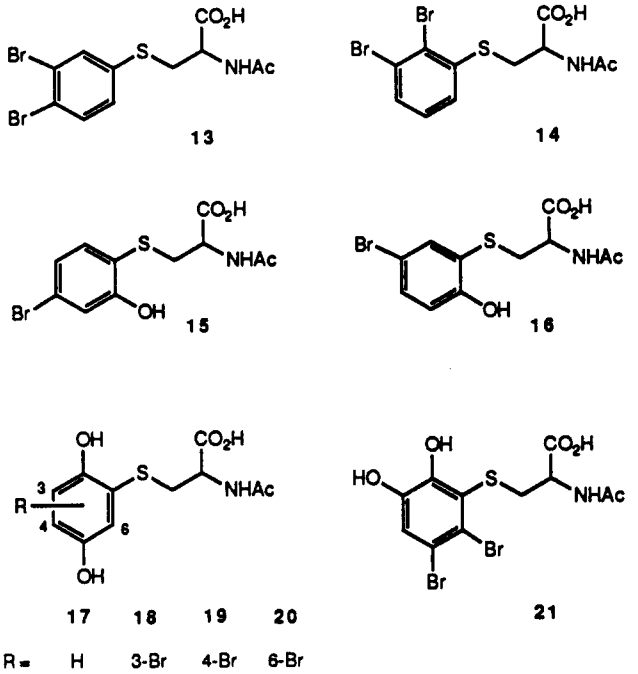
The identity of the bromobenzene metabolites that bind to proteins is not known. However, prime candidates include epoxides **5** and **6** and quinones **7** and **8**.[7-9] Although **5–8** have not been isolated per se as bromobenzene metabolites, **7** and **8** are known through their glutathione conjugates,[10,11] and **5** and **6** are known through their enzymatically formed dihydrodiols (i.e., **9** and **10**).[12,13] Epoxide **6** is also known through its enzymatically derived glutathione adduct.[14] In view of structures **5** and **12** it is surprising that neither **3** nor **4** is known as a metabolite of bromobenzene, although a recent report that mild acid treatment of **12** gives *only* **2** may explain why **3** is not a known metabolite.[15] It is also interesting that while quinones **7** and **8** react with glutathione, the corresponding mercapturic acids are not known metabolites of bromobenzene.

If metabolites **5–8** were to react with proteins, cysteinyl sulfhydryl groups should be among the likely target nucleophiles[16] and the adducts that would be formed would be *analogous* to mercapturic or premercapturic acids. Hydrolysis of either the protein adducts or the mercapturic acids should produce the corresponding S-substituted cysteines. In order to search for the "missing" *S*-(bromophenyl)mercapturic acids (i.e., **3**, **4**, and various bromoquinone-derived *S*-(bromodihydroxyphenyl)mercapturic acids) in urine, and for the corresponding cysteines from protein hydrolysates, we undertook the synthesis of mercapturic acids **2–4** and **13–21** as authentic standards. This paper describes these syntheses and the characterization of the products. In addition, we describe an alkaline degradation/permethylation procedure that efficiently converts all of these mercapturic acids to thioanisole derivatives. The latter can be identified and quantitated at extremely low levels by gas chromatography–mass spectrometry (GC–MS). This provides another method to probe for the possible covalent binding of compounds **5–8** to protein sulfhydryl groups.

Results and Discussion

Previous syntheses of aromatic mercapturic acids have relied upon two basic approaches:[17,18] In one (method A) the key step is formation of the *S*-aryl bond (a, Scheme I) while the other (method B) involves formation of the S–CH_2 bond (b, Scheme I). Method A has the advantage that the chirality of the *N*-acetylcysteine (NAC) is preserved in the product, but the traditional coupling of cuprous–cysteine complexes to aryldiazonium salts often gives low yields and complicated product mixtures, and the appropriate diazonium salts may not be readily available (viz. structures **17–21**). A variation on method



13 14 15 16 17 18 19 20 21

R = H 3-Br 4-Br 6-Br

A employs the direct (Michael) addition of NAC to quinones, but this approach can be complicated by quinone reduction at the expense of disulfide formation and by other disproportionation or coupling processes. Method B necessarily affords racemic mercapturic acids but is straightforward if the appropriate thiol is available. For our purposes we used method A to synthesize **17–21** and method B to synthesize **2–4** and **13–18**.

Synthesis of Thiophenol Derivatives. The syntheses of thiophenols corresponding to **13** and **14** began with nitration of 1,2-dibromobenzene. After separation of the 3-nitro and 4-nitro isomers, each was reduced with stannous chloride in ethanol[19] to the corresponding aniline, which was then diazotized and coupled to potassium ethylxanthate ($EtOCS_2K$).[20,21] The resulting xanthate esters (ArSCSOEt) were cleaved with KOH in aqueous ethanol and the thiols isolated by steam distillation after acidification of the reaction mixture. Similar sequences starting with either 3-bromo- or 4-bromophenol were used to prepare the thiols corresponding to **15** and **16**, except that in these cases xanthate cleavage with lithium aluminum hydride (LAH) in ether gave much better results.[21]

Reaction of 1,4-benzoquinone (**22**) with thiourea (Scheme II) yields 5-hydroxy-1,3-benzoxathiol-2-one (**24a**),[22,23] alkaline hydrolysis of which yields 2-mercaptohydroquinone (**25a**).[22] Application of this method to 2-

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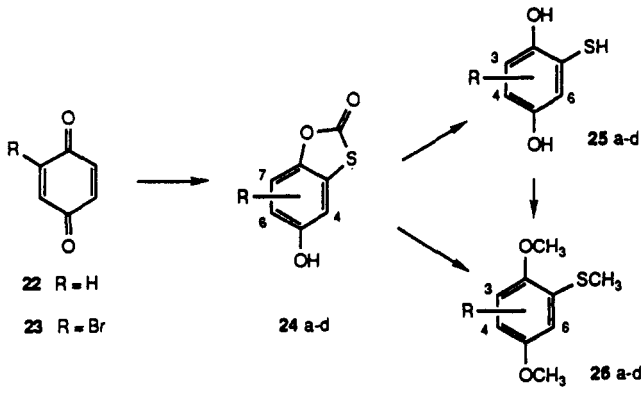
Scheme II



	R			
	a	b	c	d
24	H	7-Br	6-Br	4-Br
25	H	3-Br	4-Br	6-Br
26	H	3-Br	4-Br	6-Br

bromo-1,4-benzoquinone (**23**) yielded a mixture of isomeric benzoxathiolones in 85% yield. Column chromatography over silica gel readily separated the major isomers (**24b** ≫ **24d**), but isomer **24c** was a very minor component and was only isolated with difficulty. ^{1}H NMR spectroscopy readily distinguished the isomers on the basis of their coupling constants which were 2.4 Hz (meta), <1 Hz (para), and 8.8 Hz (ortho) for **24b**, **24c**, and **24d**, respectively. The composition of the initially formed mixture of **24b–d** (**b**:**c**:**d** ≈ 100:2:20) was determined by ^{1}H NMR and by capillary GC–MS after direct conversion to thioanisoles **26b–d** by alkaline permethylation (i.e., a modified Stekol degradation) as described below. Interestingly, reaction of **23** with thiosulfate followed by hydrolysis of the initial *S*-sulfonate ester adduct is reported to produce **25b** in low yield but as virtually the sole product.[24]

Synthesis of Aromatic Mercapturic Acids by Method B. Thiols can be activated to add to acetamidoacrylic acid (**27**) or its methyl ester (**27-Me**) under either free-radical[25] or base-catalyzed conditions. In 1948 Eiger and Greenstein[26] described the base-catalyzed addition of thiophenol to **27** to produce phenylmercapturic acid as a model for reaction of thiols with dehydropeptide linkages in proteins. Shortly thereafter, Behringer and Fackler[27] used this method to produce D,L-**1** and D,L-**2**. The presence of phenolic hydroxyl groups in some of our thiols (viz. **15–21**) was initially of some concern as a possible source of alternate nucleophiles that might react with **27** to give isomeric or even multiple addition products. However this concern proved to be needless as with mild base catalysis (piperidine in dioxane) thiols corresponding to mercapturic acids **2–4** and **13–18** generally added via their sulfur atom to **27** cleanly and in good to excellent yields. Our overall experience suggests that the success of this method is strongly dependent on the purity of the starting materials and rigorous exclusion of dioxygen during the coupling process.

Synthesis of Aromatic Mercapturic Acids by Method A. Our initial attempts at direct combination of 1,4-benzoquinone (**22**) with NAC (e.g., in aprotic media, under heterogeneous conditions with phase-transfer catalysis, or under homogeneous conditions with buffers and cosolvents) all lead to complex mixtures. Fortunately, we found that simply adding an excess of **22** in methanol to a limiting amount of NAC in distilled water lead to complete reaction in less than 30 min as judged by thin-layer chromatography (TLC) and reversed-phase high-performance liquid chromatography (HPLC). Under these conditions the major component (other than excess **22**, which could be removed by extraction with chloroform) proved to be the desired adduct **17**, accompanied by a small amount of its bright yellow quinoid counterpart (**17-Q**). After evaporation of the solvents and flash chromatography of the residue to remove excess **22** and a small amount of hydroquinone, adducts **17** and **17-Q** were eluted. Adduct **17** could be oxidized to **17-Q** by large excesses of **22**, and **17-Q** could be reduced to **17** by zinc in methanol containing trifluoroacetic acid (TFA). This redox interconversion was conveniently monitored by HPLC, which also showed that, in methanol, TFA catalyzed the conversion of **17** and **17-Q** to their methyl ester derivatives (as confirmed by their mass spectra). It was also found that, after complete reduction of **17-Q** to **17**, passage over silica gel caused the reappearance of **17-Q** as a contaminant, probably because of oxygen dissolved in the chromatography solvent.

Reaction of an excess of bromoquinone **23** with NAC as described above for **17**, followed by flash chromatographic removal of excess **23** and its corresponding hydroquinone, lead to a product mixture, which C_8 reversed-phase HPLC showed to contain three major components. After isolation of these materials by preparative HPLC (Magnum C_{18} column), ^{1}H NMR spectroscopy confirmed that they were the desired adducts **18–20** and their methyl esters. Specific structural assignments could readily be made on the basis of the coupling constants for the two ring protons. The order of elution was **20** (J = 8.8 Hz, ortho), followed by **19** (J < 1 Hz, para), **18** (J = 3 Hz, meta), and the three methyl esters in the same order. All of the mercapturic acids described herein convert to their methyl esters to varying extents upon column chromatography over silica gel with methanolic chloroform solvents.

Unlike **17**, adducts **18–20** were not readily converted to their quinone forms by air or an excess of bromoquinone **23**. However, upon being allowed to stand in acidic methanol (e.g., preparative HPLC eluate), they were converted to their methyl esters (**18-Me–20-Me**), which had much longer retention times on HPLC. This was confirmed by ^{1}H NMR and mass spectroscopic examination of the individual esters after isolation by HPLC. In a similar fashion, direct combination of NAC with 4,5-dibromo-1,2-benzoquinone afforded mercapturic acid **21**. All of the (dihydroxyphenyl)mercapturic acids were extremely hygroscopic, and removal of solvents without first drying the solutions thoroughly generally gave gummy rather than crystalline residues. With care solid residues could be obtained, but recrystallization was deemed impractical.

Alkaline Permethylation of Mercapturic Acids and Related Compounds. Mercapturic acids were discovered on the basis that alkalinization of urine containing them released thiols that were conspicuous by their odor. Stekol applied this base lability in devising gravimetric and titrimetric methods for quantitating mercapturic acids.[28] To gain greater convenience, specificity, and sensitivity, we have developed an alkaline permethylation procedure for converting mercapturic acids, the corresponding cys-

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teines, and related compounds into thioanisole derivatives that can readily be extracted, separated by GLC or HPLC, and detected by UV absorption or mass spectrometry. For example, the *S*-(bromophenyl)cysteines corresponding to **2–4** (from acid hydrolysis of the mercapturic acids) are converted to the corresponding bromothioanisoles simply by heating them in water with a large excess of K_2CO_3 and CH_3I in a screw-cap tube at 130 °C for 5 h. This same procedure also works (often under less forcing conditions) for all the aromatic thiols and benzothioxolones mentioned above, as well as for mercapturic acids **2–4** and **15–21**.

By application of this procedure with appropriate internal standards, it is possible to detect the presence of mercapturic acids related to bromobenzene metabolism (or their precursors or cysteine derivatives in protein hydrolysates) with great specificity and sensitivity and thus to confirm *independently* their putative direct detection by HPLC.[7,29] For example, C_8 reversed-phase HPLC of a "hydrophobic amino acid fraction" obtained by sequential XAD-2 and Dowex 50W-X4 chromatography of the acid hydrolysate (12 N HCl, 100 °C, 12 h) of liver proteins from rats treated with [^{14}C]bromobenzene (2.5 mmol/kg; 1.0 Ci/mol) yielded four radioactive peaks, the major two of which corresponded to (*o*- and (*m*/*p*-bromophenyl)cysteine. Alkaline permethylation of this fraction produced a mixture of all three isomeric [^{14}C]bromothioanisoles that were fully resolved by C_8 reversed-phase HPLC. By both methods we could demonstrate that covalent binding of epoxides **5** and **6** to protein sulfhydryl groups in rat liver accounts for less than 2% of the total covalently bound radioactivity. Unfortunately, mercapturic acids **17–20** (and presumably their analogous quinone–protein adducts) decompose extensively during attempted acid hydrolysis.[7] However, they undergo alkaline permethylation quite well if oxygen is rigorously excluded and an antioxidant such as ascorbate or pyrogallol is added. We are presently applying these methods to investigate the mammalian metabolism and protein covalent binding of bromobenzene both in vivo and in vitro. Full details will be published elsewhere in due course.

Experimental Section

Solvents and reagents were purchased from commercial sources and were used without further purification except as noted below. Thin-layer chromtography was carried out on glass slides 2.5 × 10 cm precoated with silica gel (Analtech). For gas chromatography 30-m flexible fused silica capillary columns with bonded phases (DB-5 and DB-Wax, J&W Scientific) were used with He carrier gas and flame ionization or mass spectrometric detection. A quadrupole GC–MS was used for conventional low-resolution electron impact spectra (EIMS, *m*/*e*) with heated probe or GC sample introduction and for desorption/chemical ionization spectra (DCIMS) with ammonia as the reagent gas. A VG-ZAB-HS high-resolution MS was used in the peak matching mode for exact mass measurements (HRMS) and for low-resolution negative ion fast atom bombardment (secondary ion) mass spectra (FABMS) with glycerol as the matrix. In reporting mass spectral data, a *double asterisk* signifies the center (^{79}Br^{81}Br) peak of the 1:2:1 cluster of a dibromo fragment ion, while a *single asterisk* signifies the ^{79}Br peak of a 1:1 isotropic doublet of a monobromo fragment ion; relative intensities are given in parentheses. ^{1}H NMR spectra were determined at either 80, 300, or 500 MHz and ^{13}C NMR spectra at 75.4 or 125.7 MHz (values measured in δ, J values in hertz). Melting points are uncorrected. The chemical as well as isomeric purity of all title compounds was judged to be ≥95% by combinations of reversed-phase HPLC, ^{1}H NMR, ^{13}C NMR, and HPLC or capillary GLC of their alkaline permethylation products.

2-Acetamido-3-[(4-bromophenyl)thio]propanoic Acid (*S*-(4-Bromophenyl)mercapturic Acid (2)). The procedure of Behringer and Fackler[27] was used. *p*-Bromothiophenol (3.1 g, 16.4 mmol) was suspended in 20 mL of freshly distilled dioxane with acetamidoacrylic acid (1.94 g, 15 mmol) (Aldrich) and 0.4 mL of piperidine, flushed with nitrogen, and refluxed for 3 h. The dioxane was then evaporated, and the residue was partitioned between ether and bicarbonate solution. The latter was neutralized with HCl, extracted with ether, and acidified to pH 1–2, after which a precipitate of crude **2** (4.15 g, 87%) formed. A portion was recrystallized from aqueous methanol. Mp: 149–151 °C (lit.[27] mp 153–154 °C). ^{1}H NMR (DMSO-d_6): 1.83 (s, 3 H); 3.18 (dd, J = 2.7, 13.5, 1 H); 3.40 (dd, J = 4.6, 13.3, 1 H); 4.40 (m, 1 H); 7.30 (d, J = 8.5, 2 H); 7.49 (d, J = 8.5, 2 H); 8.41 (d, J = 9.6, 1 H). EIMS: 317* (M^+, 9), 258* (50), 201* (5), 134 (5), 122 (45), 43 (100). FABMS: 316* ($M - H^-$, 100), 187* (70), 171* (20), 79* (32). Anal. Calcd for $C_{11}H_{12}BrNO_3S$: C, 41.52; H, 3.80; N, 4.40. Found: C, 41.75; H, 3.75; N, 4.70.

2-Acetamido-3-[(3-bromophenyl)thio]propanoic acid (*S*-(3-bromophenyl)mercapturic acid (3)) was prepared as for **2** from 3.0 g of *m*-bromothiophenol in 48% yield. Mp: 163–164 °C. ^{1}H NMR (DMSO-d_6): same as for **2** except in the aromatic region; 7.27 (t, 1 H); 7.34–7.44 (m, 2 H); 7.55 (m, 1 H). EIMS and FABMS: nearly identical with those of **2**. Anal. Calcd: see **2**. Found: C, 41.36; H, 3.65; N, 4.30.

2-Acetamido-3-[(2-bromophenyl)thio]propanoic acid (*S*-(2-bromophenyl)mercapturic acid (4)) was prepared as for **2** from 2.76 g of *o*-bromothiophenol in 47% yield. Mp: 161–162 °C. ^{1}H NMR (DMSO-d_6): same as for **2** except in the aromatic region; 7.12 (m, 1 H); 7.40 (m, 2 H); 7.61 (d, J = 7.8, 1 H). EIMS and FABMS: nearly identical with those of **2**. Anal. Calcd: see **2**. Found: C, 41.54; H, 3.69; N, 4.25.

2-Acetamido-3-[(3,4-dibromophenyl)thio]propanoic Acid (*S*-(3,4-Dibromophenyl)mercapturic Acid (13)). 1,2-Dibromobenzene (20 g, 84.8 mmol) was placed in a 100-mL flask and cooled to 0 °C, and fuming nitric acid (sp gr 1.49, 39.2 mL) was added over 5 min. The mixture was stirred at 0 °C for 15 min, poured onto 500 g of ice, and the solids were collected by filtration. TLC examination (10% EtOAc in hexanes) showed two spots above the base line: R_f = 0.58 (3,4-dibromonitrobenzene) and R_f = 0.42 (2,3-dibromonitrobenzene), in ca. 4:1 ratio. Recrystalization several times from MeOH yielded 7.45 g of the 3,4-isomer. All remaining material was combined (16 g) and chromatographed over silica gel (325 g), which yielded an additional 11.8 g of 3,4-isomer plus 4.27 g of the 2,3-isomer. The former had mp 57–58 °C (lit.[30] mp 53–54 °C), and the latter had mp 82–83 °C (lit.[31] mp 85–86 °C). Following the method of Bellamy and Ou,[19] 3,4-dibromonitrobenzene (500 mg, 1.78 mmol) was dissolved in 10 mL of absolute ethanol, $SnCl_2 \cdot 2H_2O$ (2 g, 8.9 mmol) was added, and the mixture was heated at 70 °C for 1 h and then poured onto 70 g of ice. The aqueous phase was adjusted to pH 9 with 2 N NaOH and extracted with ether, which yielded 362 mg of a brownish solid. Sublimation of the latter yielded 340 mg of 3,4-dibromoaniline as white crystals, mp 77–80 °C (lit.[32] mp 80–81 °C).

3,4-Dibromoaniline (3 g, 12 mmol) was dissolved in 30 mL of concentrated HCl with stirring, cooled to 0 °C, and diazotized by dropwise addition of $NaNO_2$ (866 mg, 12.5 mmol) in 20 mL of water. This cold diazonium reagent solution was then added over 30 min to a stirred solution of $EtOCS_2K$ (2.3 g, 14 mmol) in 30 mL of H_2O held at 60 °C (*Warning*[33]). After the solution was stirred for 2 h, a dark red-brown oil settled out. The mixture was cooled and extracted with ether, yielding 3.94 g (93%) of *O*-ethyl *S*-(3,4-dibromophenyl)xanthate. The latter was dissolved in 45 mL of EtOH, and 15 mL of H_2O, KOH (6.7 g 120 mmol)

(29) Such independent confirmation is frequently desirable if not actually *necessary*. For example, in a typical automated amino acid analysis procedure *S*-(*p*-bromophenyl)cysteine coelutes with histidine. Conversion of the former to the thioanisoles eliminates this problem in a useful way.

(30) Doyle, M. P.; Siegfried, B.; Dellaria, J. F., Jr. *J. Org. Chem.* **1977**, *42*, 2426–2430.

(31) Sihlbom, L. *Acta Chem. Scand.* **1953**, *7*, 1197–1206.

(32) Suthers, B. R.; Riggins, P. H.; Pearson, D. E. *J. Org. Chem.* **1962**, *27*, 447–451.

(33) The purpose of adding the diazonium reagent to the hot xanthate is to avoid *accumulation* of ArN=NSC(S)OEt intermediates that are *potentially* explosive. See: *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. III, p 809.

was added, and the mixture was refluxed overnight under nitrogen. After removal of the ethanol by distillation, 6 N HCl (ca. 20 mL) and zinc dust (200 mg) were added, and the mixture was steam distilled. Extraction of the distillate with ether yielded 2.1 g of a slightly brownish oil. Vacuum distillation of the latter yielded 1.88 g of 3,4-dibromothiophenol as a colorless oil: Bp: 166–171 °C (ca. 25–30 Torr). R_f = 0.48 (20% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$): 3.47 (s, SH); 7.04 (dd, J = 2.2, 8.3, 1 H); 7.36 (d, J = 8.3, 1 H); 7.54 (d, J = 2.2, 1 H). EIMS: 268** (M^+, 100), 187* (56), 108 (85).

Coupling of 3,4-dibromothiophenol (804 mg, 3 mmol) to **27** (426 mg, 3.3 mmol) was carried out as described for **2** above, yielding **13** (848 mg, 71%) as a white crystalline solid. Mp: 138–139.5 °C. R_f = 0.28 ($CHCl_3$:MeOH:HOAc = 90:10:1). ^{1}H NMR (acetone-d_6): 1.90 (s, 3 H); 3.36 (dd, J = 7.1, 14.0, 1 H); 3.54 (dd, J = 4.9, 14.1, 1 H); 4.70 (dd, J = 5.3, 7.0, 1 H); 7.34 (dd, J = 2.3, 8.4, 1 H); 7.65 (d, J = 8.4, 1 H); 7.75 (d, J = 2.0, 1 H). ^{13}C NMR (acetone-d_6): 22.51, 35.82, 52.67, 122.5, 125.4, 130.7, 134.5, 134.8, 138.7, 170.3, 171.6. EIMS: 397** (M^+, 1), 338** (4), 268** (31); 187* (25), 108 (68), 74 (24), 63 (40), 43 (100). DCIMS (NH_3): 398** (MH^+, 24), 338** (7), 318* (5), 147 (20), 130 (100). FABMS: 396** ($M - H^-$), 316* ($M - HBr^-$). DCI(NH_3)–HRMS: 395.8880; calcd for $C_{11}H_{12}{}^{79}Br_2NO_3S$, 395.8904.

2-Acetamido-3-[(2,3-dibromophenyl)thio]propanoic Acid (*S*-(2,3-Dibromophenyl)mercapturic Acid (14)). The synthesis of **14** paralleled closely that of **13**. Reduction of 2,3-dibromonitrobenzene (see above) gave 2,3-dibromoaniline in 80% yield. Mp: 45–46 °C. R_f = 0.38 (20% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$): 4.22 (br s, NH_2); 6.59 (dd, J = 3.1, 6.6, 1 H); 6.94 (m, 2 H). 2,3-Dibromoaniline was diazotized and coupled to $EtOCS_2K$ (*Warning*[33]) to give the crude xanthate as a red oil (yield 630 mg). Normal workup and chromatography over silica gel yielded the pure xanthate (313 mg, 44%) and, unexpectedly, 2,3-dibromothiophenol (243 mg, 34%), which air oxidized to the disulfide upon being allowed to stand overnight; data for the disulfide are as follows. Mp: 163–165 °C. R_f = 0.55 (20% EtOAc in hexanes). ^{1}H NMR ($CDCl_3$): 7.15 (t, J = 7.8, 1 H); 7.49 (dd, J = 1.7, 8.0, 1 H); 7.56 (dd, J = 1.7, 7.4, 1 H). EIMS: 534 ($^{79}Br_2{}^{81}Br_2 - M^+$, 5), 374** (2), 295** (22), 267** (61), 186* (100), 107 (95). The disulfide (134 mg, 0.25 mmol) was dissolved in EtOH (25 mL) and reduced with $NaBH_4$ (38 mg, 1 mmol). After 1 h water (15 mL) was added, the ethanol evaporated, and the 2,3-dibromothiophenol extracted with ether. The latter was converted to **14** (as described for isomer **13** above) and purified by reversed-phase HPLC on a C_{18} column eluted with a linear gradient of MeOH (20–40% over 20 min) in water at a flow of 1.5 mL/min. Yield: 70 mg (36%) of a white solid. Mp: 185–187 °C. R_f = 0.25 ($CHCl_3$:MeOH:HOAc = 90:10:1). ^{1}H NMR (acetone-d_6): 1.95 (s, 3 H); 3.38 (dd, J = 7.1, 13.6, 1 H); 3.56 (dd, J = 4.9, 13.5, 1 H); 4.75 (m, 1 H); 7.31 (t, J = 7.9, 1 H); 7.48 (d, J = 7.8, 1 H); 7.54 (d, J = 7.8, 1 H); 7.66 (br s, 1 H). ^{13}C NMR (acetone-d_6): 22.60, 35.41, 52.33, 124.6, 126.4, 127.0, 130.0, 131.0, 141.9, 170.4, 171.9. EIMS: 397** (M^+, 1), 374** (1), 338** (7), 268** (24), 187* (19), 108 (39), 43 (100). FABMS: 396** ($M - H^-$), 267**. HRMS: 394.8836; calcd for $C_{11}H_{11}{}^{79}Br_2NO_3S$, 394.8826.

2-Acetamido-3-[(4-bromo-2-hydroxyphenyl)thio]propanoic Acid (*S*-(4-Bromo-2-hydroxyphenyl)mercapturic Acid (15)). Nitration of *m*-bromophenol (2.8 g, 16.2 mmol) as described above for **16** gave a mixture of two nitrophenols (ca. 2:1 ratio), which were separated by chromatography on silica gel. The minor isomer, 5-bromo-2-nitrophenol, was obtained in 21% yield as yellow crystals. Mp: 42–45 °C. R_f = 0.47 ($CHCl_3$:hexanes= 1:1). Reduction of the nitrophenol with $SnCl_2{\cdot}2H_2O$[19] afforded 4-bromo-2-hydroxyaniline in 98% yield. Diazotization and coupling with $EtOCS_2K$ (*Warning*[33]) as described above afforded the xanthate as an orange oil in 49% yield. 4-Bromo-2-hydroxythiophenol (100 mg, 0.49 mmol, obtained in 78% yield after LAH reduction of the xanthate and steam distillation as above) was coupled to 70 mg of **27** as described for **2** above, which afforded **15** in 79% yield as a colorless oil after chromatography over silica gel. R_f = 0.50 (EtOAc:MeOH:HOAc = 88:10:2). ^{1}H NMR (CD_3OD): 1.94 (s, 3 H); 3.10 (dd, J = 8.1, 13.5, 1 H); 3.38 (dd, J = 4.5, 13.5, 1 H); 4.47 (m, 1 H); 6.95 (dd, J = 1.8, 8.6, 1 H); 7.00 (d, J = 1.8, 1 H); 7.26 (d, J = 8.4, 1 H). ^{13}C NMR (CD_3OD): 22.37, 35.76, 53.62, 112.1, 118.0, 123.5, 132.9, 136.4, 157.4, 173.4. EIMS: 333* (M^+, 3), 274* (8), 256* (3), 217* (5), 204* (10), 79* (100). DCIMS (NH_3): 334* (MH^+). HRMS: 332.9668; calcd for $C_{11}H_{12}NBrO_4S$: 332.9669.

2-Acetamido-3-[(5-bromo-2-hydroxyphenyl)thio]propanoic Acid (*S*-(5-Bromo-2-hydroxyphenyl)mercapturic Acid (16)). 4-Bromo-2-nitrophenol[34] (3.6 g, 16.4 mmol) was heated with $SnCl_2{\cdot}2H_2O$[19] (18.6 g, 82.4 mmol) in 35 mL of EtOH for 2 h at 70 °C. The mixture was poured onto ice (150 g), neutralized to pH 7 with $NaHCO_3$, and extracted with EtOAc, which afforded 5-bromo-2-hydroxyaniline as light gray crystals (2.3 g). Mp: 130–133 °C. The resulting aniline (3.6 g, 19.1 mmol) was diazotized and converted to the xanthate as described for **13**. R_f = 0.27 (60% $CHCl_3$ in hexanes). ^{1}H NMR ($CDCl_3$): 1.30 (t, 3 H); 4.57 (q, 2 H); 5.90 (br s, 1 H); 6.90 (d, J = 4.5, 1 H); 7.2–7.5 (m, 2 H).

The xanthate (1.65 g, 5.6 mmol) was dissolved in 15 mL of Et_2O and the resultant mixture added slowly to a suspension of LAH (0.8 g, 21 mmol) in 70 mL of Et_2O. After the mixture was allowed to reflux overnight under nitrogen, water was cautiously added followed by sufficient concentrated HCl to dissolve the aluminum residues, and the ether layer was separated and evaporated. Dilute HCl and a small amount of zinc metal were added, and the residue was steam distilled under nitrogen to yield 5-bromo-2-hydroxythiophenol as colorless crystals. Mp: 54–57 °C. R_f = 0.79 (EtOAc:$CHCl_3$ = 1:1). ^{1}H NMR ($CDCl_3$ + trace D_2O): 6.83 (d, J = 8.6, 1 H); 7.31 (dd, J = 2.4, 8.7, 1 H); 7.57 (d, J = 2.4, 1 H). EIMS: 206* (M^+, 100), 177* (15), 125 (70), 97 (95).

The resulting thiophenol (170 mg, 0.83 mmol) was then coupled with **27** (120 mg, 0.92 mmol) as described above for **2**. The crude product was chromatographed over silica gel with 1–50% EtOH in $CHCl_3$ to afford **16** in 68% yield as a colorless oil. R_f = 0.50 (EtOAc:MeOH:HOAc = 88:10:2). ^{1}H NMR (acetone-d_6): 1.94 (s, 3 H); 3.23 (dd, J = 7.8, 13.7, 1 H); 3.41 (dd, J = 3.9, 14.0, 1 H); 4.65 (m, 1 H); 6.88 (d, J = 9.0, 1 H); 7.31 (dd, J = 2.4, 9.3, 1 H); 7.57 (d, J = 2.4, 1 H). ^{13}C NMR (CD_3OD): 23.36, 35.87, 53.63, 119.5, 120.6, 123.4, 124.0, 136.3, 159.4, 173.3, 173.5. EIMS: 333* (M^+, 1), 274* (1), 204* (20), 125 (20), 97 (18), 43 (100). HRMS: 332.9665; calcd for $C_{11}H_{12}N{}^{79}BrO_4S$, 332.9669.

***S*-(2,5-Dihydroxyphenyl)mercapturic Acid (17) by Method B.** Benzoquinone (**22**; 10.9 g, 0.1 mol) was dissolved in 80 mL of glacial acetic acid and the resultant mixture added dropwise to a solution of thiourea (9.2 g, 0.11 mol) in 100 mL of 2 N HCl with stirring at room temperature. Subsequently 5 mL of concentrated HCl was added, a white precipitate formed, and the entire mixture was heated at 90–95 °C for 1 h. Upon cooling to 0 °C, white crystals of 5-hydroxy-1,3-benzothioxol-2-one (**24a**) formed and were collected by filtration, washed with ice water, and dried. Mp: 172–173 °C (lit.[23] mp 174–175 °C). R_f = 0.50 (10% EtOH in $CHCl_3$). Thioxolone **24a** (8.8 g, 52.3 mmol) was dissolved in an excess of thoroughly deaerated (N_2 purged) 2 N NaOH and the mixture heated to reflux under nitrogen for 1 h. The mixture was cooled, acidified with H_2SO_4, saturated with Na_2SO_4, and extracted with ether. The ether solution was dried and evaporated to yield 7.4 g of 2-mercaptohydroquinone as white crystals. Mp: 113–114 °C (lit.[22] mp 118 °C). EIMS: 142 (100), 113 (24), 109 (17), 81 (30). Coupling of the thiol to acetamidoacrylic acid (**27**) as described for **2** above gave **17** as a yellowish solid in 47% yield (27% after recrystallization from ethyl acetate with addition of hexane). Mp: 138–139 °C. R_f = 0.38 (EtOH:$CHCl_3$:HOAc = 40:60:1). ^{1}H NMR (acetone-d_6): 1.94 (s, 3 H); 3.14 (dd, J = 7.3, 13.7, 1 H); 3.33 (dd, J = 5.5, 13.7 1 H); 4.60 (m, 1 H); 6.67 (dd, J = 2.5, 9.0, 1 H); 6.76 (d, J = 9.3, 1 H); 6.93 (d, J = 3, 1 H). ^{13}C NMR (acetone-d_6): 22.58, 37.31, 53.18, 117.0, 120.8, 121.0, 151.0, 151.5, 171.9, 172.0. EIMS: 271 (M^+, 3), 212 (3), 194 (4), 155 (7), 144 (88), 43 (100). DCIMS (NH_3): 272 (MH^+, 11), 142 (25), 130 (100). FABMS: 270 ($M - H^-$, 90), 141 (100). HRMS: 271.0509; calcd for $C_{11}H_{13}NO_5S$, 271.0515.

2-Acetamido-3-[(2,5-dihydroxyphenyl)thio]propanoic Acid (*S*-(2,5-Dihydroxyphenyl)mercapturic Acid (17)) by Method A. Recrystallized benzoquinone (810 mg, 7.5 mmol) was dissolved in 90 mL of MeOH and the resultant mixture added to NAC (490 mg, 3.0 mmol) in 10 mL of H_2O. After the solvents were removed in vacuo, ethanol (50 mL) and silica gel (5 g) were added, and the mixture was dried on a rotary evaporator. The product

(34) Rinehart, K. L., Jr.; McMillan, M. W.; Witty, T. R.; Tipton, C. D.; Shield, L. S. *Bioorg. Chem.* **1977**, *6*, 353–369.

adsorbed on the silica gel was placed atop a silica gel column (50 g) and eluted with 400 mL of $CHCl_3$ (to remove quinone and hydroquinone) and 400 mL of 30% MeOH in $CHCl_3$. Fractions containing **17** were pooled and evaporated to give a brownish residue (800 mg). Examination of this material by HPLC on a C_{18} reversed-phase column (4.6 × 250 mm) eluted at 1.5 mL/min with a linear gradient of 10–90% MeOH in water (constant 0.1% TFA) over 30 min showed only two main peaks, **17** (R_t = 9.75 min) and its quinoid form **17-Q** (R_t = 12.9 min). Addition of excess benzoquinone caused an increase in the **17-Q**/**17** ratio. Conversely, **17-Q** was readily reduced to **17** by granular zinc in aqueous methanol containing a small amount (1–3%) of TFA. After being allowed to stand in acidic methanol, both **17** and **17-Q** were converted to their methyl esters (confirmed by mass spectrometry). On silica TLC, **17** and **17-Q** did not separate well if at all; spots containing **17-Q** were yellow, and spots containing **17** became yellow upon exposure to air for 30–90 min (or faster if exposed to iodine vapors).

2-Acetamido-3-[(2,3-dibromo-5,6-dihydroxyphenyl)thio]propanoic Acid (*S*-(2,3-Dibromo-5,6-dihydroxyphenyl)mercapturic Acid (21)). 4,5-Dibromocatechol (536 mg, 2 mmol) was dissolved in CH_2Cl_2 (54 mL) and the resultant mixture added to Ag_2CO_3 on Celite[35] (5.7 g, 10 mmol of Ag). The resulting slurry was stirred for 6 h and filtered and the filtrate evaporated to yield a red crystalline solid (382 mg, 71%). The latter was immediately dissolved in ethanol (21 mL), and a solution of NAC (234 mg, 1.43 mmol) in 7 mL of H_2O was added. After being allowed to stand at 4 °C overnight, the solvents were removed in vacuo to yield 602 mg of a yellowish gum. A portion of this was subjected to preparative HPLC on a C_{18} reversed-phase column (4.6 × 250 mm, eluted at 1.5 mL/min with a gradient of methanol in water increasing from 50% to 90% over 10 min and then held at 90%). Four main peaks were observed (254-nm detection): 5.4 min (unknown), 8.1 min (adduct **21**), 11.2 min (4,5-dibromocatechol), and 14.4 min (unknown). From 100 mg of crude reaction mixture, 24 mg of **21** was collected; it was initially a colorless oil, but upon standing, it became slightly yellow and solidified. Upon heating above 130 °C, it decomposed without melting. R_f = 0.12 (MeOH:$CHCl_3$:HOAc = 10:90:1). ^{1}H NMR (80 MHz, acetone-d_6): 1.95 (s, 3 H); 3.23 (dd, 2 H); 4.54 (m, 1 H); 6.54 (br s, 2 H); 7.16 (s, 1 H); 7.57 (d, NH). DCIMS (NH_3): 430** (MH^+, 14), 350* (7), 147 (17), 130 (100), 112 (17), 60 (92), 43 (31). HRMS: 427.8779; calcd for $C_{11}H_{11}{}^{79}Br_2NO_5S$, 427.8803.

Bromo-5-hydroxy-1,3-benzothioxol-2-ones 24b–d. 2-Bromohydroquinone (1.29 g, 6.8 mmol, purified by chromatography over silica gel with $CHCl_3$) was dissolved in 2% H_2SO_4 (30 mL), and $NaClO_3$ (550 mg, 5.2 mmol) and V_2O_5 catalyst[36] (30 mg) were added. After stirring overnight, the yellow reaction was extracted with CH_2Cl_2, evaporation of which yielded 1.11 g (87%) of 2-bromo-1,4-benzoquinone (**23**). The latter was immediately dissolved in 7 mL of glacial acetic acid and the mixture added to 0.49 g (6.4 mmol) of thiourea in 12 mL of 2 N HCl. After 30 min the yellow had disappeared, 5 mL of concentrated HCl was added, and the mixture was refluxed for 90 min. After the mixture was allowed to stand at 25 °C overnight, a white precipitate formed. The latter was collected by filtration, washed with cold H_2O, and dried, yielding 1.13 g of a white solid (85% of theory). TLC examination of this material (3% EtOH in $CHCl_3$) showed primarily three spots plus some polar material near the origin. These materials were separated by silica gel column chromatography and characterized as follows.

7-Bromo-5-hydroxybenzothioxol-2-one (24b). Mp: 183.5–184.5 °C. R_f = 0.42 (3% EtOH in $CHCl_3$). ^{1}H NMR (acetone-d_6): 3.0 (br s, 1 H); 7.06 (d, J = 2.4, 1 H); 7.18 (d, J = 2.4, 1 H). EIMS: 246* (M^+, 40); 218* (25); 190* (10), 139 (100), 111 (60). Anal. Calcd for $C_7H_3BrO_3S$: C, 34.03; H, 1.22. Found: C, 34.38; H, 1.10.

6-Bromo-5-hydroxybenzothioxol-2-one (24c). Mp: 143–144 °C. R_f = 0.52 (3% EtOH in $CHCl_3$). ^{1}H NMR (acetone-d_6): 2.87 (br s, OH); 7.36 (s, 1 H); 7.62 (s, 1 H). EIMS: 246* (M^+, 30), 218* (8), 190* (35), 111 (70), 53 (100).

4-Bromo-5-hydroxybenzothioxol-2-one (24d). Mp: 169–169.5 °C. R_f = 0.49 (3% EtOH in $CHCl_3$). ^{1}H NMR (acetone-d_6): 3.0 (br s, OH); 7.06 (d, J = 9.0, 1 H); 7.28 (d, J = 9.0, 1 H). EIMS: 246* (M^+, 40), 218* (14), 190* (44), 139 (100), 111 (44), 53 (32).

(Bromodihydroxyphenyl)mercapturic Acids 18–20 by Method A. Bromoquinone **23** (from oxidation of 1.13 g of 2-bromohydroquinone as described above) was suspended in 90 mL of MeOH and the mixture added to 290 mg (2.4 mmol) of NAC. After 2 h the solvents were removed and the residue was dissolved in EtOH, mixed with 3 g of silica gel, and evaporated to dryness. This dried residue was placed atop a silica gel column (40 g) and eluted with 600 mL of 1% EtOH in $CHCl_3$. Further elution with 800 mL of 10% EtOH in $CHCl_3$ yielded 700 mg of a mixture of adducts. Rechromatography of this material over C_{18} bonded-phase silica gel with 20% MeOH in 0.1% aqueous TFA gave 670 mg of a slightly brownish solid. HPLC examination of this material (performed on a 10-μm C_8 reversed-phase column, 4.6 × 250 mm, eluted at 1.5 mL/min with 20% MeOH in 0.1% aqueous TFA) indicated six principal peaks. After repetitive injections, each of these peaks was isolated in sufficient quantity (15–60 mg) for unambiguous characterization as the three mercapturic acids **18–20** and their corresponding methyl esters. The retention times for the acids were 14.5 min (**20**), 17.1 min (**19**), and 19.2 min (**18**); for the methyl esters the retention times were 28.6 min (**20**-Me), 32.2 min (**19**-Me), and 33.7 min (**18**-Me). The ^{1}H NMR spectra of these acids and esters showed the characteristic *N*-acetylcysteine protons (viz. **2–4** and **15–17**, above), the methyl ester group (a sharp singlet at 1.86 ppm), and the two expected aromatic hydrogens with coupling constants of 8.8 Hz (ortho, **20** and **20**-Me), 3.0 Hz (meta, **18** and **18**-Me), and <1 Hz (para, **19** and **19**-Me).

Methyl 2-Acetamido-3-[(3-bromo-2,5-dihydroxyphenyl)thio]propanoate (Methyl *S*-(3-Bromo-2,5-dihydroxyphenyl)mercapturate (18-Me)). ^{1}H NMR (DMSO-d_6): 1.85 (s, 3 H); 3.05 (dd, J = 13.7, 9.3, 1 H); 3.18 (dd, J = 13.7, 5.4, 1 H); 3.58 (s, 3 H); 4.39 (ddd, J = 5.4, 7.8, 9.3, 1 H); 6.68 (d, J = 3.0, 1 H); 6.80 (d, J = 3.0, 1 H); 8.48 (d, J = 7.3, 1 H); 8.75 (br s, 2 H). ^{13}C NMR (DMSO-d_6): 22.3, 33.5, 51.4, 52.1, 111.9, 115.9, 117.3, 125.6, 144.3, 151.2, 169.5, 170.9. HRMS: 362.9780; calcd for $C_{12}H_{14}{}^{79}BrNO_5S$, 362.9776.

Methyl 2-Acetamido-3-[(4-bromo-2,5-dihydroxyphenyl)thio]propanoate (Methyl *S*-(4-Bromo-2,5-dihydroxyphenyl)mercapturate (19-Me)). ^{1}H NMR (DMSO-d_6): virtually identical with **18**-Me except in the aromatic region; 6.81 (s, 1 H); 6.92 (s, 1 H). ^{13}C NMR (DMSO-d_6): 22.5, 33.0, 51.5, 52.0, 107.5, 117.9, 118.5, 120.5, 146.8, 149.8, 169.4, 171.1. HRMS: 362.9790; calcd (same as for **18**-Me).

Methyl 2-Acetamido-3-[(6-bromo-2,5-dihydroxyphenyl)thio]propanoate (Methyl *S*-(6-Bromo-2,5-dihydroxyphenyl)mercapturate (20-Me)). ^{1}H NMR (DMSO-d_6): virtually identical with **18**-Me except in the aromatic region; 6.74 (d, J = 8.8, 1 H); 6.87 (d, J = 8.8, 1 H). ^{13}C NMR (DMSO-d_6): 22.31, 34.94, 51.95, 51.99, 115.0, 117.0, 117.1, 120.1, 147.4, 151.7, 169.2, 171.1. HRMS: 362.9780; calcd (same as for **18**-Me).

2-Acetamido-3-[(3-bromo-2,5-dihydroxyphenyl)thio]propanoic Acid (*S*-(3-Bromo-2,5-dihydroxyphenyl)mercapturic Acid (18)) by Method B. Thioxolone **24b** (180 mg, 0.73 mmol) was dissolved in 10 mL of THF and the resultant mixture added to 75 mg of LAH in 5 mL of THF. After the reaction refluxed overnight, the reaction was cooled and water was added cautiously. The THF was evaporated, and the residue partitioned between 6 N HCl and ether to give 160 mg (100%) of thiol **24b** as a white solid. R_f = 0.52 (10% EtOH in $CHCl_3$). ^{1}H NMR (acetone-d_6): 6.82 (s, 2 H). EIMS: 220* (87, M^+), 141 (30), 113 (50), 97 (15), 53 (100). This thiol (100 mg, 0.45 mmol) was immediately coupled to acetamidoacrylic acid (130 mg, 1.0 mmol), as described for the synthesis of **17**. After removal of the dioxane the residue was dissolved in $NaHCO_3$ solution and extracted with ether. The aqueous phase was then acidified to pH 1–2 and extracted with ethyl acetate. The latter was dried ($MgSO_4$) and evaporated, and the residue was chromatographed over silica gel. This afforded 30 mg (21%) of **18** as a slightly yellowish gum that would not crystallize even after further purification by preparative HPLC. ^{1}H NMR (DMSO-d_6): 1.86 (s, 3 H); 3.00 (dd, J = 12.9, 8.5, 1 H); 3.21 (dd, J = 13.4, 4.6, 1 H); 4.33 (m, 1 H); 6.68 (d, J = 3, 1 H); 6.78 (d, J = 3, 1 H); 8.23 (d,

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J = 7.8, 1 H). EIMS: 349* (M^+, 1), 290* (1), 272* (1), 233* (2), 220* (4), 141 (4), 111 (5), 43 (100). HRMS: 348.9622; calcd for $C_{11}H_{12}BrNO_5S$, 348.9618.

Alkaline Permethylation of Mercapturic Acids. A mixture of compounds **2–4** (0.95 mg, 3 μmol each) was dissolved in 1.5 mL of water in a 13 × 100 mm culture tube and purged briefly with nitrogen. A 3-g sample (21.7 mmol) of solid K_2CO_3 and 0.5 mL (8.0 mmol) of CH_3I were then added, and the tube was sealed with a Teflon-lined screw cap. The tube was then immersed up to the level of the liquid meniscus in an oil bath kept at 130 °C behind a safety shield in a hood. The CH_3I (lower layer) refluxed vigorously, giving good mixing, while the upper portion of the culture tube served as an air-cooled condenser. After 5 h the oil bath was lowered away, the tube was allowed to cool and opened cautiously, and the contents were extracted with *n*-pentane (4 × 2 mL). After the volume of the extracts was adjusted and an internal standard (2,3-dichloronitrobenzene was convenient) was added, the yields of the bromothioanisole isomers were determined by reversed-phase HPLC with a C_8 column (10 μm, 4.6 × 250 mm) eluted with 50% methanol at 1.5 mL/min. On the basis of comparison to authentic standards, the retention times for *o*-, *p*-, and *m*-bromothioanisole were 19.2, 23.8, and 26.0 min, respectively. Peak integration, with correction for differences in absorption at 254 nm, indicated the yeilds of the three isomers were 76, 73, and 76%, respectively.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of compounds **13–17** and the methyl esters of compounds **18–20** and ^1H NMR spectrum of compound **21** (9 pages). Ordering information is given on any current masthead page.

Linearly Fused vs Bridged Regioselection in the Intramolecular 1,3-Diyl Trapping Reaction

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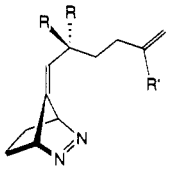
Received July 21, 1989

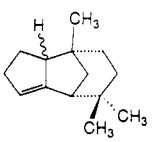
The intramolecular diyl trapping reaction can now be used to obtain synthetically useful quantities of either bridged or linearly fused cycloadducts in a *selective* manner and *by design*. Bridged cycloadducts arise by intercepting the triplet diyl, while linearly fused products can be produced from either the singlet or the triplet. When an electron-withdrawing group is attached to the diylophile, the singlet diyl leads selectively to fused cycloadducts. On the other hand, the presence of a large alkyl group attached to the internal carbon of the diylophile affords bridged cycloadducts selectively from cycloaddition with the triplet. Four diazenes, **4–7**, differing only in the electronic and steric properties of the substituent located on the internal carbon of the diylophile, were studied. The diyl trapping reactions were conducted using ca. 1 mM solutions of diazene in THF at reflux for periods of 3–4 h; cycloadduct yields ranged from 68% (beginning with the dimethyl ketal **7**) to 98% (from keto diazene **4**). To determine the origin of the bridged cycloadducts, the effect of oxygen upon the product distribution was examined. The results show that the rate of the intramolecular triplet diyl cycloaddition is slower than the rate of the intermolecular reaction of the triplet with oxygen. The rate of triplet intramolecular cycloaddition can be *estimated* to be less than 4×10^6 to 4×10^7 s^{-1}.

Introduction

Several years ago, we became intrigued by the observation that unlike all previously conducted intramolecular diyl trapping reactions, the major product produced in the cycloaddition of the diyl derived from diazene **1** was the bridged (**2**), rather than the linearly fused regioisomer (**3**).[3] We were particularly interested in learning how to obtain *either* product *selectively*, and *by design*. That objective has been achieved and the results of our investivation are described below.



1, R = R' = CH_3
4, R = H, R' = $COCH_3$
5, R = H, R' = CH_2OH
6, R = H, R' = $CH(OCH_3)_2$
7, R = H, R' = $CCH_3(OCH_3)_2$



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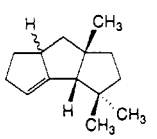

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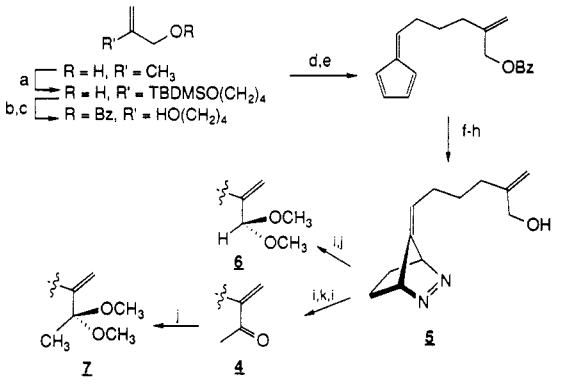
(1) Taken, in part, from the Ph.D. Dissertation of M.R.M., UCSB, 1989.

(2) In part; I.D.D. began study of the chemistry of diazene **5**: Dannecker-Doerig, I. M.S. Dissertation, UCSB, 1985.

(3) (a) Little, R. D.; Carroll, G. L.; Petersen, J. L. *J. Am. Chem. Soc.* **1983**, *105*, 928. (b) Little, R. D. *Chem. Rev.* **1986**, *86*, 875. (c) the linearly fused tricyclopentanoids invariably display a signal at ca. 3.1 ppm which is characteristic for the hydrogen located at the A,B ring junction.

Scheme I. Preparation of Diazenes[a]



[a] (a) *n*-BuLi, TMEDA, −78 to 25 °C, 17 h; $Br(CH_2)_3OTBDMS$, THF, −78 °C, 10 min, 78%; (b) BzCl py, DMAP, CH_2Cl_2, 0–25 °C, 1 h, 96%; (c) HF, CH_3CN, 25 °C, 2.5 h, 96%; (d) PCC, Celite, CH_2Cl_2, 25 °C, 2 h, 85%; (e) CpH, Et_2NH, CH_3OH, 0–25 °C, 2.5 h; HOAc, 91%; (f) $(NCO_2Me)_2$, CH_2Cl_2, 4 °C, 8 h; (g) $(NCO_2K)_2$, HOAc, CH_2Cl_2, 0 °C, 1.5 h, 94% (two steps); (h) KOH, EtOH, reflux, 1.5 h; $K_3Fe(CN)_6$, H_2O, 0 °C, 97%; (i) Dess–Martin periodinane, CH_2Cl_2, 97%; (j) $HC(OCH_3)_3$, PPTS, CH_3OH; (k) MeLi, Et_2O, −78 °C, 74%.

Synthesis of Diyl Precursors

Four diazenes, **4–7**, differing only in the electronic and steric properties of the substituent located on the internal carbon of the diylophile, were studied. Each was syn-

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