The Synthesis of Two Phenacetin Metabolites

A. FOCELLA, P. HESLIN, AND S. TEITEL
Chemical Research Department, Hoffmann–La Roche Inc., Nutley, New Jersey 07110

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A new metabolite of phenacetin was identified as 4-hydroxy-3-methylthioacetanilide (**11**). The synthesis of **11** and the isomeric 4-hydroxy-2-methylthioacetanilide (**4**) as well as that of the previously reported phenacetin metabolite 3-[(5-acetamido-2-hydroxyphenyl)thio]alanine (**18**) is described.

Un nouveau métabolite de phénacétine a été identifiée comme 4-hydroxy-3-méthylthioacétanilide (**11**). La synthèse de **11** et celle du 4-hydroxy-2-méthylthioacétanilide isomérique sont décrites, aussi bien que celle du métabolite préalablement rapporté de phénacétine, 3-[(5-acétamido-2-hydroxyphényl)thio]alanine (**18**).

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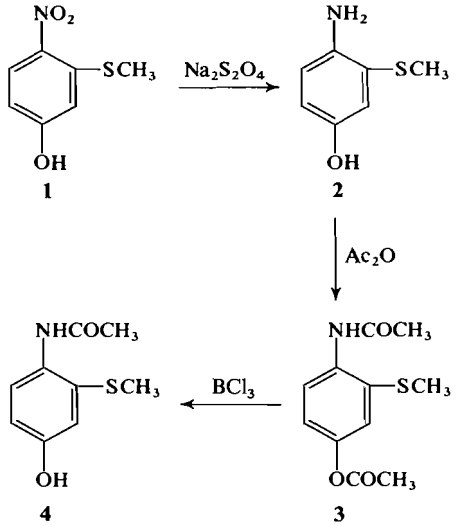
In connection with a recent study on the metabolism of phenacetin (*p*-ethoxyacetanilide), a new metabolite isolated from dog urine[1] (1) exhibited n.m.r. and mass spectra[2] compatible with either 4-hydroxy-2-methylthioacetanilide (**4**) or 4-hydroxy-3-methylthioacetanilide (**11**). It therefore became necessary, in order to establish unequivocally the structure of this metabolite, to synthesize in an unambiguous manner both isomers.

The 2-methylthio isomer **4** was prepared (Scheme 1) as follows. Reduction of the known 4-nitrothioether **1** (2) with sodium hydrosulfite in alkaline solution provided the substituted aniline **2**. Conversion of **2** with acetic anhydride into the acetoxyacetanilide **3** followed by *O*-deacetylation with boron trichloride afforded 4-hydroxy-2-methylthioacetanilide (**4**).



SCHEME 1. Synthesis of 4-hydroxy-2-methylthioacetanilide (**4**).

The isomeric 4-hydroxy-3-methylthioacetanilide (**11**) was prepared (Scheme 2) from the key intermediate **9** which was obtained by two methods. Diazotization of the aminonitrophenol **5** in fluoboric acid gave the known (3)[3] diazooxide **6** which was converted by a Sandmeyer reaction with sodium thiomethylate to the thioether **7**. Subsequent reduction of the nitro group with sodium hydrosulfite afforded **9**. Alternatively, treatment of the thiophenol **8** with diazotized sulfanilic acid followed by sodium hydrosulfite reduction yielded **9** more directly. Treatment of **9** with acetic anhydride in the presence of *p*-toluenesulfonic acid gave a separable mixture of the desired hydroxyacetanilide **11** and its *O*-acetoxy derivative **10**. Saponification of **10** with aqueous alkali also afforded **11**.

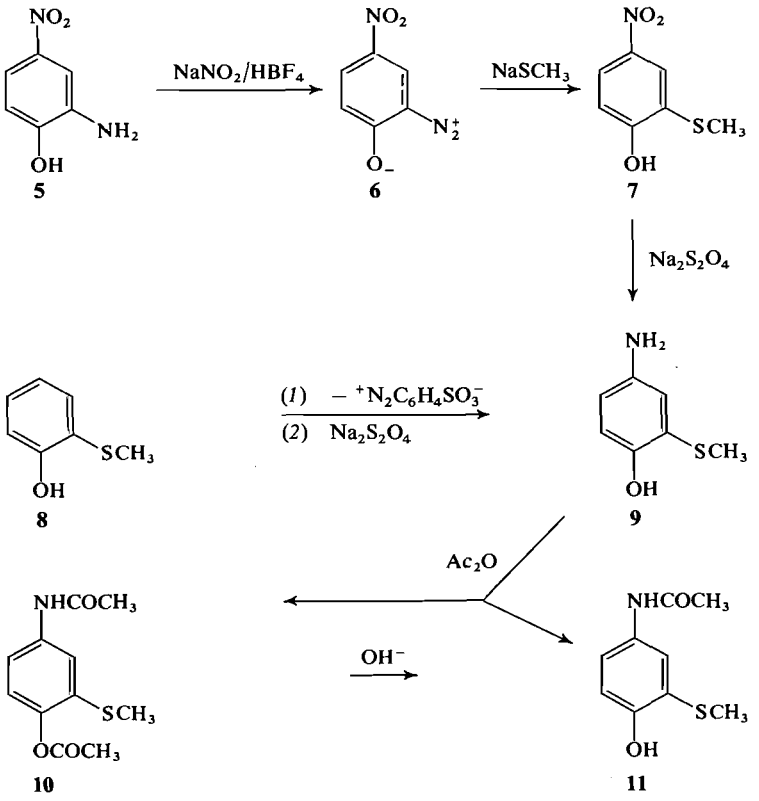
A comparison of the pertinent n.m.r. and m.s. data for the metabolite and the synthesized isomers **4** and **11** (Table 1) indicates that the metabolite and compound **11** are identical. The data for the metabolite and **4** are unlike; there are large differences in the chemical shifts of the aromatic protons in the n.m.r. and in the relative intensities for the three major peaks in the mass spectra.

Having established the structure of this metabolite as 4-hydroxy-3-methylthioacetani-

[1]Made available by Dr. A. Klutch of Burroughs Wellcome & Co.

[2]We are grateful to Dr. F. Vane and Mr. R. Pitcher of our Physical Chemistry Department for these determinations.

[3]Previously characterized only by a thermal decomposition study.

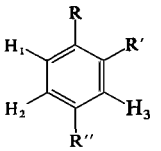


SCHEME 2. Synthesis of 4-hydroxy-3-methylthioacetanilide (**11**).

TABLE 1. Comparison of n.m.r. and mass spectrum data for the metabolite (1) with **4** and **11**

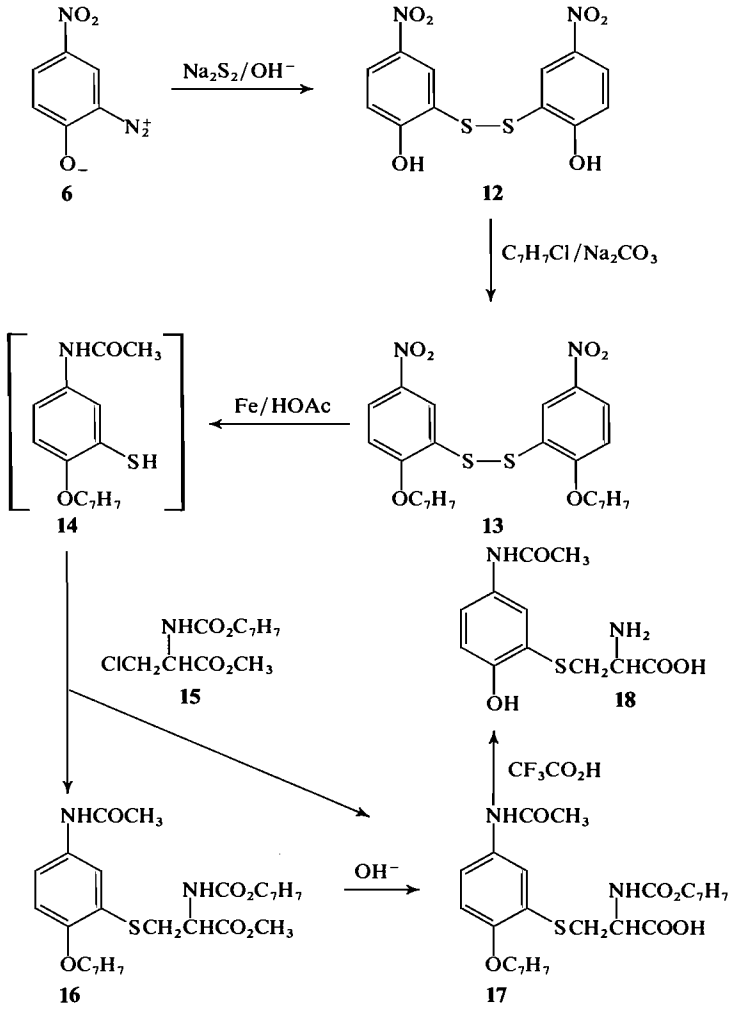
	Nuclear magnetic resonance (δ)*						Mass spectrum (m/e)‡		
			Aromatic protons†			NH or			
Compound	$COCH_3$	SCH_3	H_1	H_2	H_3	OH	197 (M^+]	155 [$M-C_2H_2O$]	140 [$M-C_2H_2O$, CH_3]
Metabolite (1)	2.15	2.35	6.90	—	~7.70	~6.48	100	70–72	68–75
4	2.20	2.38	7.85	6.65	6.83	5.06	75–100	88–97	94–100
11	2.16	2.36	6.93	7.22	7.67	6.41	100	70–90	67–92

*Obtained in chloroform-*d* solutions with a Varian HA-100 spectrometer using a C-1024 time averaging computer.
†H_1, H_2, and H_3 correspond to the following protons:



H_2 of the metabolite was obscured by a chloroform impurity peak.
‡The low resolution mass spectra were obtained with a CEC 21–110 mass spectrometer operating at 70 eV. The large range in the relative intensities observed for the three major peaks in the mass spectra of the metabolite and the two isomers **4** and **11** may be a temperature effect. Intensities are given in % base peak.

lide (**11**), it appeared reasonable that another phenacetin metabolite previously identified as *S*-(1-acetamido-4-hydroxyphenyl)cysteine (**4**) was probably related to **11** and therefore should be 3-[(5-acetamido-2-hydroxyphenyl)thio]alanine (**18**). To prove this hypothesis, we synthe-



SCHEME 3. Synthesis of 3-[(5-acetamido-2-hydroxyphenyl)thio]alanine (**18**).

sized **18** as follows (Scheme 3). A Sandmeyer reaction carried out by treating the diazo-oxide **6** with sodium polysulfide provided the disulfide **12** which was converted into the di-*O*-benzyl derivative **13**. Reaction of **13** with iron[4] in acetic acid effected simultaneously reduction, acetylation, and cleavage of the disulfide to afford the unstable thiophenol **14**. *S*-Alkylation of **14** in ethyl acetate[5] with α-benzyloxycarbonylamino-β-chloropropionic acid methyl ester (**15**), obtained from 3-chloroalanine methyl ester, yielded a separable mixture of the methyl ester **16** and a minor amount of the corresponding acid **17**. Saponification of **16** also provided the acid **17** which was selectively debenzylated and decarbobenzylated with trifluoroacetic acid to the desired compound **18**.

Comparison of the physical data reported for the metabolite *S*-(1-acetamido-4-hydroxyphenyl)cysteine (4) and those exhibited by **18** (Table 2) indicates their similarity in these respects. However, we could not unequivocally establish their identity since a sample of the metabolite was not available for a more direct correlation.

[4]In contrast to iron, zinc reductively cleaved the disulfide **13** in very poor yield.

[5]Attempts to *S*-alkylate the unstable **13** in other conventional solvents gave mainly the corresponding disulfide.

TABLE 2. Physical data of metabolite *S*-(1-acetamido-4-hydroxyphenyl)cysteine and **18**

Compound	Melting point (°C)	Ultraviolet absorption (λ_{max}, nm) 0.1 *N* HCl	0.1 *N* KOH	Infrared absorption (μ) Amine	Amide	Paper chromatography* (R_f) Ascending	Descending
Metabolite (4)	187–188 (needles)	203 243 295	270 313	6.2	6.0 6.5	0.70	0.28
18	192–193 (needles)	200 240 292	268 312	6.2	6.0 6.5	0.68	0.26

*Paper chromatography was carried out according to the conditions described (4) using Whatman No. 1 chromatography paper employing butan-1-ol : acetic acid : water (4:1:1) for ascending development and phenol : water : concentrated ammonium hydroxide (800:200:1) for descending development and detected with ninhydrin.

Experimental

All melting points (uncorrected) were taken in open capillary tubes in a Thomas–Hoover melting point apparatus. The i.r. spectra were determined with a Beckman IR-5 or IR-9 recording spectrophotometer and the u.v. spectra with a Carey 14 spectrophotometer. The n.m.r. spectra were obtained with either a Varian A-60A, Varian HA-100, or a Jeolco C-60H spectrometer using tetramethylsilane as internal standard.

4-Amino-3-methylthiophenol (2)

To a stirred solution of 3-methylthio-4-nitrophenol (**1**) (27 g, 0.146 mol) in 10% NaOH (250 ml) at 60°, sodium hydrosulfite (75 g, 0.430 mol) was cautiously added. The mixture was stirred and heated at 95° for 40 min, allowed to cool to room temperature, and the crystalline precipitate collected to give 20 g (88%) of **2**, m.p. 145–148°. An analytical sample prepared from ethyl acetate exhibited: m.p. 148–150°; i.r. (KBr) 3330, 3300 (NH_2), 2780–2570 cm^{-1} (associated OH of phenol); n.m.r. (DMSO-d_6) δ 2.31 (s, 3, CH_3), 4.40 (br. s, 2, NH_2), 6.66 (br. s, 1, H_2), 6.53 (br. s, 2, $H_{5,6}$), 8.50 (s, 1, OH).

Anal. Calcd. for C_7H_9NOS: C, 54.17; H, 5.84. Found: C, 54.25; H, 5.55.

4-Acetoxy-2-methylthioacetanilide (3)

A mixture of the substituted aniline **2** (15 g, 0.0965 mol), *p*-toluenesulfonic acid (15 g), and acetic anhydride (200 ml), was stirred overnight at room temperature. The excess acetic anhydride was distilled under reduced pressure and the residue partitioned between water and ethyl acetate. The organic phase was washed successively with water, two 100-ml portions of 1% NaOH, 0.1 *N* HCl, and evaporated under reduced pressure. The crystalline residue of **3** weighed 15.5 g (67%) and melted at 103–106°. An aliquot prepared from ether exhibited: m.p. 100–102°; i.r. ($CHCl_3$) 3380 (NH), 1760 (phenolic ester), 1685, 1510 cm^{-1} (amide); n.m.r. ($CDCl_3$) δ 2.20, 2.30, 2.40 (s, 9, 3 CH_3), 7.00 (d of d), 7.20 (d), 8.30 (d) (ABX, 3, J_{AB} meta 2.5, J_{AX} ortho 7 Hz, aromatic), 8.05 (br. s, 1, NH).

Anal. Calcd. for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.47. Found: C, 55.17; H, 5.65.

4-Hydroxy-2-methylthioacetanilide (4)

To a solution of the acetanilide **3** (2 g, 84 mmol) in methylene chloride (100 ml) at −78° was slowly added with stirring a methylene chloride solution (21 ml) containing boron trichloride (10 mmol). The mixture was allowed to stir 4 h at room temperature, methanol (4 ml) added dropwise, washed with water and 1 *N* hydrochloric acid, and extracted with 2% sodium hydroxide. Neutralization of the alkaline solution with dilute hydrochloric acid precipitated 1.1 g (67%) of crystalline **4**, m.p. 147–149°. A sample crystallized from acetone gave: m.p. 148–149°; i.r. (KBr) 3230 (OH + NH), 1626, 1520 cm^{-1} (amide); n.m.r. (DMSO-d_6) δ 1.95, 2.32 (s, 6, 2 CH_3), 6.61 (d of d), 6.48 (d), 6.98 (d) (ABX, 3, J_{AB} meta 2.5, J_{AX} ortho 7 Hz, aromatic), 8.95, 9.33 (s, 2, NH + OH); mass spectrum (70 eV) *m/e* (relative intensity) 197 (75–100), 155 (88–97), 140 (94–100).

Anal. Calcd. for $C_9H_{11}NO_2S$: C, 54.80; H, 5.67. Found: C, 54.94; H, 5.85.

4-Nitrobenzene-2-diazo-1-oxide (6)

To a stirred suspension of 20 g (0.130 mol) of 2-amino-4-nitrophenol (**5**) (20 g, 0.13 mol) in 48% fluoboric acid (50 mol), maintained at 4°, a cold solution of sodium nitrite (9 g, 0.13 mol) in water (18 ml) was added dropwise. The mixture was stirred for 30 min at room temperature, the solid collected, washed once with cold fluoboric acid solution (10 ml), then with two 15-ml portions of cold water, and sucked dry. The solids were suspended in acetone (200 ml), collected, and dried under P_2O_5 to yield 15 g (70%) of **6**, m.p. 120° (with violent decomposition[3]). A sample recrystallized from water and acetone had m.p. 120°; u.v. max (2-propanol) 238 (ε 11 620), 259 (12 610), 324 (11 350), 400 (6650), 490–500 nm (1000) (sh); n.m.r. (MeOD + DCl) δ 7.22 (d, 1, *J* ortho 10 Hz, aromatic), 8.60 (d of d, 1, *J* meta 3, *J* ortho 10 Hz, aromatic), 9.27 (d, 1, *J* meta 3 Hz, aromatic).

Anal. Calcd. for $C_6H_3N_3O_3$: C, 43.63; H, 1.83; N, 25.45. Found: C, 43.62; H, 1.68; N, 25.45.

4-Hydroxy-3-methylthionitrobenzene (7)

To a vigorously stirred mixture of sodium hydroxide (20 g), copper powder (3 g), and methanethiol (25 g, 0.52 mol) in water (70 ml) was added slowly[6] at about 4° a suspension of the diazo-oxide **6** (15 g, 0.091 mol) in ice water (60 g). The reaction mixture was stirred at room temperature for 1 h, filtered, and the cake washed with 5% sodium hydroxide (50 ml). The combined filtered material was acidified to p*H* 5 with 6 *N* hydrochloric acid and the resulting precipitate was filtered, washed with water, and purified by means of semi-dry column chromatography (5) as follows.

[6]Ether was added as necessary to control the foaming.

The solids were dissolved in ethyl acetate (100 ml), 35 g of chromatographic grade silica gel (0.05–0.02 mesh) deactivated with 8% of water was added and the mixture evaporated to dryness. The residue was deposited on top of a column (20 × 3 in.) packed with 300 g of the deactivated silica gel, followed by the addition of a 1:1 mixture of ethyl acetate and benzene (70 ml). The column was eluted with benzene (200 ml) and the eluate evaporated to give 7 g (42%) of **7** of m.p. 58–62°. An analytical sample prepared from petroleum ether showed: m.p. 64–65°;[7] i.r. (KBr) 3330 (bonded OH of phenol), 1510, 1335 cm^{-1} (NO_2); n.m.r. ($CDCl_3$) δ 2.44 (s, 3, CH_3), 8.43 (d), 8.16 (d of d), 7.09 (d) (ABX, 3, J_{AB} meta 2.5, J_{BX} ortho 9 Hz, aromatic), 7.30 (s, 1, OH).

Anal. Calcd. for $C_7H_7NO_3S$: C, 45.38; H, 3.81. Found: C, 45.63; H, 4.13.

*4-Hydroxy-3-methylthioaniline (**9**)*

(a) Preparation from 7

A stirred solution of 35 g (0.190 mol) of the thioether **7** (35 g, 0.19 mol) in 10% sodium hydroxide (200 ml) was heated to 45–50° and sodium hydrosulfite (76 g, 0.44 mol) was added cautiously in two portions. The mixture was heated on a steam bath for 25 min and allowed to cool to room temperature. The crystals were filtered, washed with water, and dried in a vacuum oven at 60° to give 22 g (75%) of **9**, m.p. 170–175°. An analytical sample prepared from ethyl acetate exhibited: m.p. 190–192°; i.r. (KBr) 3356, 3300 (NH_2), 2500 cm^{-1} (associated phenolic OH); n.m.r. (DMSO-d_6) δ 2.28 (s, 3, CH_3), 4.28 (br, 2, NH_2), 6.42, (d of d), 6.25 (d), 6.53 (d) (ABX, 3, J_{AB} meta 2.5, J_{AX} ortho 8 Hz, aromatic), 8.40 (br. s, 1, OH).

Anal. Calcd. for C_7H_9NOS: C, 54.17; H, 5.84; N, 9.03. Found: C, 54.51; H, 5.92; N, 8.93.

*(b) Preparation from **8***

To a stirred mixture of 2-(methylmercapto)phenol (**8**) (20 g, 0.14 mol) and sodium hydroxide (32 g) in water (210 ml) was added at 7–12° a diazonium salt suspension prepared by diazotization (6) of sulfanilic acid (41 g, 0.21 mol). The stirred mixture was allowed to warm to room temperature, heated to 45–50° and sodium hydrosulfite (130 g, 0.74 mol) was added in two portions. The mixture was stirred and heated on a steam bath for 45 min, allowed to cool to room temperature, and the resulting crystalline precipitate collected and recrystallized from ethyl acetate to give 16.4 g (74%) of **9**, identical in mixed m.p. and n.m.r. with **9** obtained from **7**.

*4-Acetoxy-3-methylthioacetanilide (**10**)*

A mixture of 4-hydroxy-3-methylthioaniline (**9**) (15.5 g, 0.1 mol), *p*-toluenesulfonic acid (15.5 g), and acetic anhydride (200 ml) was stirred at room temperature overnight. The excess acetic anhydride was distilled under reduced pressure and the residue partitioned between water and ethyl acetate. The organic phase was washed with water, with two 75-ml portions of 1% sodium hydroxide (this alkaline solution contained **11**), and with water until neutral p*H*. The ethyl acetate solution was dried (Na_2SO_4), the solvent evaporated, and the residue crystallized from ethyl acetate to give 14.4 g (58%) of **10**: m.p. 146–147°; i.r. (KBr) 3280 (NH), 1765 (ester CO), 1653, 1550 (amide), 1212, 1180 cm^{-1} (C—O—C of ester).

[7]Dimorphic; another crystalline form exhibited m.p. 93–94°.

Anal. Calcd. for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.47. Found: C, 55.53; H, 5.54.

*4-Hydroxy-3-methylthioacetanilide (**11**)*

*(a) Preparation from **9***

The alkaline phase from the preparation of **10** was neutralized with dilute hydrochloric acid and the resulting crystals, recrystallized from ethyl acetate, gave 2.8 g (14%) of **11**: m.p. 130–131°; i.r. ($CHCl_3$) 3230 (OH, NH) 2400 (associated OH of phenol), 1690, 1570 cm^{-1} (amide); u.v. max (2-propanol) 234 (ε 19 850), 301 nm (4450); u.v. max (0.1 *N* HCl) 227 (ε 18 650), 294 nm (3750); u.v. (0.1 *N* KOH) 222 (ε 19 730), 265 (9320); 309 nm (6400); n.m.r. ($CDCl_3$) δ 2.16 (s, 3, CH_3CO), 2.32 (s, 3, CH_3S), 7.22 (d of d), 6.93 (d), 7.67 (d) (AMX, 3, J_{AM} ortho 8, J_{AX} meta 2.5 Hz, aromatic); mass spectrum (70 eV) *m/e* (relative intensity) 197 (100), 155 (70–90), 140 (67–92).

Anal. Calcd. for $C_9H_{11}NO_2S$: C, 54.88; H, 5.62. Found: C, 54.89; H, 5.50.

*(b) Preparation from **10***

A suspension of the acetanilide **10** (6 g, 0.025 ml) in 2.5% sodium hydroxide (100 ml) was stirred at 50° for 30 min, cooled, and extracted with two 75-ml portions of ethyl acetate. The aqueous layer was acidified to p*H* 5 with dilute hydrochloric acid, re-extracted with two 100-ml portions of ethyl acetate, dried (Na_2SO_4), and the extracts evaporated. The residue was crystallized from ethyl acetate to give 3.0 g (61%) of **11**, m.p. 129–131°; identical in mixed m.p. with **11** obtained from **9**.

*Bis(2-hydroxy-5-nitrophenol)disulfide (**12**)*

To a vigorously stirred solution, maintained at 0–10°, of sodium polysulfide (prepared by dissolving a mixture of sodium sulfide monohydrate (130 g, 1.35 mol) and sulfur powder (17 g, 0.53 mol) in 40% sodium hydroxide (50 ml) and water (140 ml) and heating on a steam bath) was added portionwise over 45 min a suspension of the diazo-oxide **6** (55.9 g, 0.39 mol) in ice water (150 ml). The resulting solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was cooled, acidified with concentrated hydrochloric acid, ethyl acetate (600 ml) added, stirred for 45 min, and the amorphous solids filtered. The organic extract was concentrated to about 350 ml and stirred overnight with a solution of sodium carbonate (53 g, 0.5 mol) in water (200 ml). The precipitate was filtered, washed with two 100-ml portions of ethyl acetate, and dried (Na_2SO_4) to give 90 g (68.5%) of **12** as the disodium salt which was used for the next step. An aliquot was acidified with 1 *N* hydrochloric acid, extracted with ethyl acetate, and the extracts evaporated. The residue was crystallized from ethyl acetate to give yellow crystals of **12**: m.p. 174–176°; i.r. (KBr) 3375 (OH), 1602, 1474 (aromatic), 1510, 1330 cm^{-1} (NO_2); n.m.r. (MeOD) δ 6.93 (d), 8.00 (d of d), 8.36 (d) (AMX, 3, J_{AM} meta 2.5, J_{MX} ortho 9 Hz, aromatic).

Anal. Calcd. for $C_{12}H_8N_2O_6S_2$: C, 42.35; H, 2.37; N, 8.23; S, 18.84. Found: C, 42.67; H, 2.70; N, 8.00; S, 18.86.

*Bis(2-benzyloxy-5-nitrophenyl)disulfide (**13**)*

A mixture of the crude disodium salt of **12** (60 g, 0.155 mol), sodium carbonate (40 g, 0.38 mol), and benzyl chloride (60 g, 0.48 mol) in ethanol (600 ml) was stirred and refluxed for 24 h. The yellow crystals were filtered, washed with cold ethanol (35 ml), and then with water until the washings were neutral to litmus paper and dried to give

60 g (74.4%) of **13**, m.p. 172–174°. An analytical sample prepared from ethyl acetate exhibited: m.p. 176–177°; u.v. max (2-propanol) 207 (ε 42 400), 225 (21 500) (infl), 258 (21 800), 293 (18 350), 330 nm (10 800) (sh); i.r. (KBr) 1596, 1474 (aromatics), 1510, 1335 (NO_2), 1270 cm^{-1} (C—O—C).

Anal. Calcd. for $C_{26}H_{20}N_2O_6S_2$: C, 59.99; H, 3.87; N, 5.38; S, 12.32. Found: C, 60.12; H, 3.89; N, 5.32; S, 12.43.

α-Benzyloxycarbonylamino-β-chloropropionic Acid Methyl Ester (15)

To a vigorously stirred mixture of 3-chloroalanine methyl ester hydrochloride (30 g, 0.173 mol), ethyl acetate (300 ml), and saturated sodium bicarbonate solution (100 ml) was added benzyl chloroformate (60 g, 0.35 mol). The mixture was maintained at 5° and rendered slightly alkaline by the addition of saturated sodium bicarbonate as needed. The mixture was stirred at room temperature for 2 h, cooled to 5°, and a solution of piperidine (25 g, 0.29 mol) in water (50 ml) was added dropwise. The reaction was allowed to warm to room temperature, the organic phase separated, washed with two 75-ml portions of 3 *N* hydrochloric acid, then with water, dried (Na_2SO_4), and evaporated under reduced pressure. The oily residue was crystallized from petroleum ether to give 35 g (75%) of **15**, m.p. 51–52°. An analytical sample prepared from petroleum ether gave: m.p. 51–52°; i.r. (KBr) 3445 (NH), 1760 (ester CO), 1730 (urethane), 1520 cm^{-1} (amide).

Anal. Calcd. for $C_{12}H_{14}ClNO_4$: C, 53.05; H, 5.19; Cl, 13.05. Found: C, 53.04; H, 5.14; Cl, 13.15.

rac-3-[(5-Acetamido-2-benzyloxyphenyl)thio]-N-benzyloxycarbonylalanine Methyl Ester (16)

A mixture of the di-*O*-benzyl derivative **13** (14 g, 0.027 mol), glacial acetic acid (200 ml), and iron powder (30 g) was stirred and refluxed for 3 h, cooled to 60°, and filtered through a thin pad of Hyflo. The cake was washed with acetic acid (50 ml) and the combined filtrates were evaporated under reduced pressure. The residue was partitioned between ethyl acetate (120 ml) and 2% hydrochloric acid (50 ml) and the organic extract washed with water (30 ml), dried (Na_2SO_4), decanted and redried (anhydrous $CaSO_4$). To the ethyl acetate solution in a nitrogen atmosphere, the methyl ester **15** (15 g, 0.055 mol) was added. The mixture was stirred and slowly heated to reflux under N_2 while maintaining the p*H* between 7 and 8 by addition of a solution of 17% of sodium methoxide in methanol as needed. After stirring and refluxing for a total of 6 h, the mixture was allowed to stand overnight at room temperature. The resulting crystals were filtered, washed with water, and dried to give 12 g of **16**, m.p. 133–135°. The organic filtrate was extracted with two 100 ml portions of sodium carbonate solution and the aqueous extract acidified to precipitate 2.4 g (9%) of **17**, m.p. 188–192°. The spent ethyl acetate solution was evaporated under reduced pressure and the residue crystallized from ethyl acetate to give an additional 3 g (total 52.1%) of crude **16**, m.p. 132–134°. An analytical sample of **16** prepared from ethyl acetate exhibited: m.p. 141–142°; i.r. (KBr) 3300, 3280 (NH), 1730 (ester), 1717 (urethane), 1650, 1530 cm^{-1} (amide); n.m.r. (DMSO-d_6) δ 2.03 (s, 3, CH_3CO), 3.67 (s, 3, CH_3O), 4.22 (m, 1, CH), 5.06, 5.16 (s, 4, 2 CH_2O), 6.90–7.70 (m, 13, aromatic), 9.84 (s, 1, NH).

Anal. Calcd. for $C_{27}H_{28}N_2O_6S$: C, 63.76; H, 5.55; N, 5.51. Found: C, 63.62; H, 5.57; N, 5.46.

rac-3-[(5-Acetamido-2-benzyloxyphenyl)thio]-N-benzyloxycarbonylalanine (17)

A mixture of rac-3-[5-acetamido-2-benzyloxyphenyl)-thio]-*N*-benzyloxycarbonylalanine methyl ester (**16**) (5 g, 0.01 mol), 5% sodium hydroxide (100 ml) was heated on a steam bath for 20 min, Norit added, and the solution filtered hot through a thin pad of Hyflo. The cake was washed with 5% sodium hydroxide (20 ml) and the combined filtrates neutralized with 1 *N* hydrochloric acid. The mixture was allowed to stand for 4 h, the precipitate filtered, washed with water, and dried to give 3.46 g (70%) of **17**, m.p. 188–190°. An analytical sample prepared from ethyl acetate exhibited: m.p. 198–200°; i.r. (KBr) 3320 (NH, OH), 2700–2600 (acid OH), 1730–1705 (acid CO), 1670, 1550 cm^{-1} (amide); n.m.r. (DMSO-d_6) δ 2.03 (s, 3, CH_3CO), 3.28 (d, 2, $J = 6$ Hz, CH_2S), 4.12 (m, 1, CH), 5.04, 5.14 (s, 4, $2CH_2O$), 6.90–7.70 (m, 13, aromatic), 9.78 (s, 1, NH).

Anal. Calcd. for $C_{26}H_{26}N_2O_6S$: C, 63.14; H, 5.30; N, 5.66. Found: C, 63.36; H, 5.37; N, 5.67.

rac-3-[(5-Acetamido-2-hydroxyphenyl)thio]alanine (18)

A mixture of the acid **17** (4 g, 0.008 mol), trifluoroacetic acid (100 ml), and boron trifluoride etherate (12 drops) was stirred at reflux under N_2 for 4 h and then at room temperature overnight. The solvent was evaporated under reduced pressure, the oily residue dried by azeotropic distillation with benzene, the semi-solid residue was suspended in warm ethyl acetate (20 ml), and the mixture stored at room temperature for 2 h. The supernatant solution was decanted, and the residue crystallized from a mixture of methanol (20 ml) and benzene (50 ml) to give 0.78 g of **18**, m.p. 184–186° dec. From the mother liquor, a second crop of 0.25 g, m.p. 178–182° (dec.) (total 47%) was obtained. An analytical sample prepared from a mixture of water and ethanol exhibited: m.p. 192–193°; u.v. max (0.1 *N* KOH) 268 (ε 9580), 312 nm (6200); i.r. (KBr) 3200 (OH, NH), 3070–2600 (NH_3^+), 1650 (amide), 1610 (COO^-), 1500 (amide), 1410 cm^{-1} (COO^-); n.m.r. (DMSO-d_6) δ 1.99 (s, 3, CH_3CO), 2.80–3.40 (m, 2, CH_2), 6.82, (d), 7.35 (d of d), 7.59 (d) (AMX, 3, J_{AM} ortho 8.5, J_{MX} meta 2.5 Hz, aromatic), 9.84 (s, 1, NH); mass spectrum, thermal decomposition.

Anal. Calcd. for $C_{11}H_{14}N_2O_4S$: C, 48.88; H, 5.22; N, 10.36; S, 11.86. Found: C, 48.55; H, 5.08; N, 10.11; S, 11.67.

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