30469-83-1; *p*-phenylbenzhydryl methyl ether, 30469-84-2; *m*-phenylbenzhydryl methyl ether, 30470-00-9.

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Selective Cyanylation of Sulfhydryl Groups. II. On the Synthesis of 2-Nitro-5-thiocyanatobenzoic Acid

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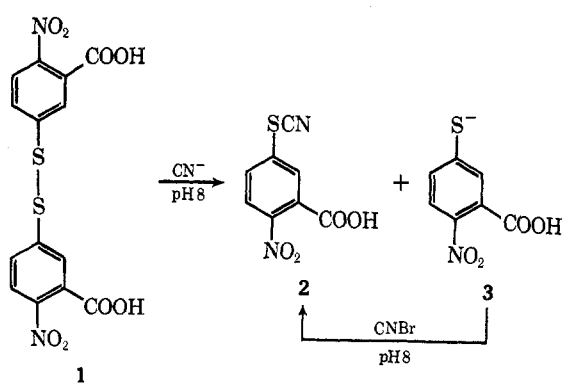
We have recently described a method for the selective cyanylation of sulfhydryl groups under mild conditions, employing 2-nitro-5-thiocyanatobenzoic acid (NTCB, **2**).[1] The reagent was shown to be particularly useful for the reversible blocking of cysteine residues with the cyano group in peptides and proteins and for radioactive labeling of proteins at cysteine residues when using ^{14}C-NTCB. The reagent is also a promising tool for the selective nonenzymatic cleavage of peptide chains at cysteine residues, since *N*-acyl-β-thiocyanoalanines were shown to undergo cyclization to labile *N*-acyl-2-iminothioazolidine rings with subsequent cleavage of the *N*-acyl function.[2,3]

NTCB was originally prepared[1] by treatment of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent,[4] **1**) with NaCN, forming besides **2** an equimolar amount of the thionitrobenzoate **3**. The latter was removed from the product mixture by treatment with bromoacetyl-cellulose, thus shifting the equilibrium toward quantitative completion of the reaction.

We now describe an improved synthesis of NTCB giving twice as high yield of the product as in the previous method. Instead of removing **3** from the reaction mixture, it is also converted into the desired product by treatment with an equimolar amount of cyanogen bromide. Thus 1 mol of **1** gives 2 mol of **2** in practically quantitative yields.



Both steps of the synthesis can be followed by the appearance and disappearance of the characteristic color of **3** [λ_{max} 412 mμ (ε 13,600)].[5]

For obtaining a quantitative yield of **2**, the presence of excess cyanide is necessary also in the second step. Thus, when the pure thiol **3** (prepared by reducing the disulfide **1** with β-mercaptoethanol) was treated with cyanogen bromide without addition of cyanide salt, the yield of the thiocyanate was only 58%, the rest of the thiol (42%) being converted into the disulfide **1**. (The products were separated and determined by a quantitative paper electrophoretic method, described in the Experimental Section.) The disulfide was probably formed *via* the thiocyanate, by reaction with the still unreacted thiol. Indeed, when the thiol **3** was treated with excess thiocyanate **2** at pH 7–8, the disulfide **1** was formed. These findings point to the existence of equilibrium 1. It is therefore concluded that the presence of excess cyanide during the CNBr reaction shifts the equilibrium to the left, in the direction of the desired thiocyanate.

$$RSCN + RS^- \rightleftharpoons RSSR + CN^- \quad (1)$$

This interpretation is supported by two recent reports on the reaction of cyanogen bromide with thiols. Foye, *et al.*,[6] found that the reaction of a thiol with cyanogen bromide (in 2:1 molar ratio) provides a synthesis of disulfides, whereas Kottke, *et al.*,[7] reported that treatment of thiols with "nascent cyanogen bromide" (excess cyanide followed by dropwise addition of bromine) afforded the corresponding thiocyanates in good yields. Under the experimental conditions of the latter reaction, excess cyanide was present continuously during the synthesis, probably effecting equilibrium 1 as suggested above.

Under the appropriate conditions, the reaction of aromatic thiols with cyanogen bromide seems advantageous over common routes to aryl thiocyanates, such as reacting aryl halides or diazonium salts with metal thiocyanates, since the aryl thiocyanates obtained by these methods are often accompanied by the corresponding isothiocyanates.[8] This contamination was also observed in our earlier attempts to prepare **2** from either 5-chloro-2-nitrobenzoic acid or diazotized 5-amino-2-nitrobenzoic acid.

Experimental Section

Melting points were determined with a Fisher–Johns apparatus and were uncorrected. Infrared spectra were recorded on a Perkin-Elmer 237 spectrophotometer, uv spectra on a Cary 15 spectrophotometer, and mass spectra on a MAT CH4 mass spectrophotometer. Paper electrophoresis was run in a Savant high-voltage electrophoresis apparatus Model LT-48A using pyridine acetate buffer of pH 3.5. Descending paper chromatography was run with 25:6:25 1-butanol–acetic acid–water. Whatman No. 1 paper was used for both electrophoresis and chromatography.

2-Nitro-5-thiocyanatobenzoic Acid (2).—To a 150-ml aqueous solution containing 7.5 g of $KHCO_3$ and 2.0 g (31 mmol) of KCN, 3.0 g (7.5 mmol) of 5,5′-dithiobis(2-nitrobenzoic acid) (**1**,

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(3) For review see T. F. Spande, B. Witkop, Y. Degani, and A. Patchornik, *Advan. Protein Chem.*, **24**, 98 (1970).

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(6) W. O. Foye, A. M. Hebb, and J. Mickles, *J. Pharm. Sci.*, **56**, 292 (1967).

(7) K. Kottke, F. Friedrich, and R. Pohloudek-Fabini, *Arch. Pharm. (Weinheim)*, **300**, 583 (1967).

(8) R. G. R. Bacon in "Organic Sulfur Compounds," Vol. I, N. Kharasch, Ed., Pergamon Press, Oxford, London, New York, Paris, 1961, p 306.

Aldrich Chemical Co.) was added with magnetic stirring. After 30 min, a freshly prepared 3% solution of cyanogen bromide (Eastman Organic Chemicals) in water was slowly added (10 min) to the stirred deep orange solution, until the color was completely discharged; 27 ml was thus consumed (110% of the theoretical). After decreasing the pH to 5 by the dropwise addition of glacial HOAc, excess cyanide was removed by bubbling a stream of nitrogen through the soution for 12 hr. Upon acidification to pH 2.3 with 6 *N* HCl a white solid crystallized out. After ice cooling the mixture, the solid was filtered, washed with cold water, and air-dried, yield 3.42 g (94%) of chromatographically and electrophoretically pure product, mp 248°. Recrystallization from ethanol gave 2.95 g (81% overall) of pale yellow plates, mp 249°. The product proved to be the half potassium salt of **2**.

Anal. Calcd for $KH(C_8H_3N_2O_4S)_2$: C, 39.50; H, 1.45; N, 11.52; S, 13.80; K, 8.04. Found: C, 40.10; H, 1.46; N, 11.39; S, 13.57; K, 7.50.

Titration with 0.1 *N* $HClO_4$ in HOAc using methyl violet as indicator gave a neutral equivalent of 484 (theory 486).

The free acid was prepared by following an analogous procedure but using NaCN in 0.5 *M* Tris acetate[9] buffer of pH 8.2 instead of KCN in $KHCO_3$ solution. The free acid crystallized out upon acidifying the final solution, yield 3.10 g (92%) of chromatographically and electrophoretically pure product, mp 160–161°. Recrystallization from ethyl acetate–petroleum ether gave 2.66 g (79%) of pale yellow prisms, mp 162–163°. Free **2** was also obtained from its half salt by suspending the latter in dilute HCl, followed by extraction with ethyl acetate, evaporation, and recrystallization from ethyl acetate–petroleum ether.

Anal. Calcd for $C_8H_4N_2O_4S$: C, 42.87; H, 1.80; N, 12.50; S, 14.28. Found: C, 42.95; H, 1.75; N, 12.45; S, 14.06.

Attempts to determine the neutral equivalent of the acid by visual titration with NaOMe in benzene–methanol were unsuccessful because the basic titrant decomposed the thiocyanate group, forming **3**.

Both **2** and its half salt showed an identical single uv-absorbing spot on paper electrophoresis (60 V/cm, 90 min, 30 cm from the origin toward the anode) and paper chromatography (R_f 0.83), which turned yellow (forming **3**) on spraying with an aqueous methanolic solution of Na_2S. **1** behaves similarily, but its electrophoretic mobility is 1.17 of that of **2** and its R_f is 0.92, in the above systems, respectively. Both **2** and its half salt showed the following spectral features: identical uv spectra, λ_{max} (0.1 *M* phosphate buffer pH 7.3) 293 mμ (ε 8000); ir (KBr) sharp SCN band 2170 cm^{-1}; mass spectra (70 eV) heaviest peak at *m/e* 224, corresponding to the molecular ion of the free acid.

Upon treatment of 10^{-4} *N* solutions of either **2** or its half salt in 0.1 *M* phosphate buffer pH 7.3 with excess β-mercaptoethanol, **3** was formed instantaneously in 99 and 102% yields, respectively, as determined by its characteristic absorption.[4,5]

5-Mercapto-2-nitrobenzoic Acid (3).—To a solution of 1.00 g (2.5 mmol) of **1** in 50 ml of 0.5 *M* Tris hydrochloride buffer pH 8.0, 5 ml (71 mmol) of β-mercaptoethanol was added. After 5 min the solution was acidified to pH 1.5 by the addition of 6 *N* HCl. By ice cooling the solution for 24 hr, orange crystals were formed which were filtered, washed with diluted HCl, and vacuum-dried over P_2O_5: yield 0.57 g (57%); mp 137–138°; uv λ_{max} (0.1 *M* phosphate buffer containing 0.005 *M* EDTA) 412 mμ (ε 13,660) (lit.[4,5] 13,600); the absorbancy of the solution remained unchanged after addition of either **1** or β-mercaptoethanol, showing that the product was free of traces of either β-mercaptoethanol or **1**, respectively; molecular weight mass spectrum (70 eV) showed the molecular ion peak at *m/e* 199; iodometric titration (in 50% aqueous HOAc) gave a value of 200.1.

Anal. Calcd for $C_7H_5NO_4S$: C, 42.22; H, 2.53; N, 7.03; S, 16.08. Found: C, 42.34; H, 2.45; N, 7.16; S, 15.96.

Reaction of 5-Mercapto-2-nitrobenzoic Acid (3) with Cyanogen Bromide.—To a solution of 40 mg (0.2 mmol) of **3** in 4.5 ml of Tris hydrochloride buffer pH 8.0, 1.0 ml of 3% aqueous solution of CNBr (0.28 mmol) was added dropwise, the initial deep orange color of the thiolate thereby changing to a pale yellow color of the formed disulfide. Ten-μl samples of the mixture were subjected to paper electrophoresis under the above conditions. Two uv-absorbing spots, corresponding to **1** and **2**, were detected. Each spot was cut into thin strips and eluted for 45 min in 5.0 ml of 0.4 *M* β-mercaptoethanol in 0.1 *M* phosphate buffer pH 7.3, thereby forming yellow **3**. The latter was subsequently determined by its absorption at 412 mμ. (By this procedure, the recovery of **3** from chromatographed control samples of pure **1** and **2** was 98–100%.) The results showed that the yields of **1** and **2** obtained by the CNBr reaction were 42 and 58%, respectively.

(9) Tris(hydroxymethyl)aminomethane.

Registry No.—**2**, 30211-77-9; **2** half K salt, 30344-83-3; **3**, 15139-21-6.

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The Stereochemistry of the 2,2′-Methylenedicycloalkanones

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We recently have been studying potential synthetic routes to C_{20}–C_{26} macrocycles with emphasis on the inclusion of polyketonic functionality. During this initial investigation, we prepared 2,2′-methylenedicyclopentanone (**1**) and -dicyclohexanone (**2**), both of which exist as two separable diastereomers, whose configurations have been tentatively assigned either on the lack of a dipole moment for **1**[1] or a tedious reduction–resolution sequence for **2**.[2] We herein describe a simple procedure for the configurational assignment of these and related δ diketones.

The base-catalyzed condensation of paraformaldehyde with cyclopentanone or cyclohexanone gave *dl*-**1** and *meso*-**1** or *dl*-**2** and *meso*-**2**, respectively.[3] The isomer stability had been previously established, since thermal epimerization of each isomer is negligible at 120°,[2] and each can be easily derivatized without loss of stereochemical integrity.[1]

Upon repetitious recrystallization of **1**, a single pure isomer (mp 71°) can be isolated, along with a lower melting (mp 38°) component. Each isomer was treated with perbenzoic acid in CH_2Cl_2 in the presence of sodium bicarbonate generating (90%) the corresponding lactones. Since this reaction is known to proceed stereospecifically with retention of configuration, the identical stereochemistry of the resultant lactones is thus established.[4]

Without purification, the crude lactones were converted (>80%) to the 2,2-dimethyldioxane dimethyl esters. The 71° melting isomer of **1** was transformed stereospecifically to a *single* substituted dioxane (*dl*-**5**), while the 38° melting component of **1** was shown by glc analysis to be a mixture which was comprised of 39% of *dl*-**5** and 61% of the isomeric dioxane (*meso*-**5**).

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