

# Synthesis of $\omega$ -(4-Aminophenylsulfonamido)alkyl Disulfides and Thiosulfates and their Activity against Dihydropteroate Synthetase from Sulfanilamide-Resistant *Neisseria gonorrhoeae*

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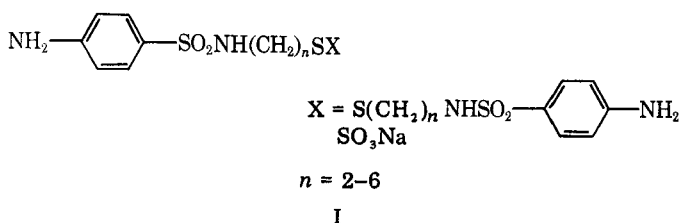
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**Abstract** □ A series of  $\omega$ -(4-aminophenylsulfonamido)alkyl disulfides and  $\omega$ -(4-aminophenylsulfonamido)alkanethiosulfates was synthesized from the reaction of *p*-acetamidobenzenesulfanil chloride and either the aminoalkyl disulfide dihydrobromide or the aminoalkyl bromide hydrobromide followed by sodium thiosulfate. Several of the compounds showed inhibitory activity against dihydropteroate synthetase isolated from a sulfanilamide-resistant strain of *Neisseria gonorrhoeae* of the same order of activity as that of sulfanilamide. An increase in the hydrophobic nature of the sulfanilamide structure did not increase inhibitory activity against this enzyme.

**Keyphrases** □  $\omega$ -(4-Aminophenylsulfonamido)alkyl—disulfides and thiosulfates, synthesis, activity against dihydropteroate synthetase, sulfanilamide-resistant *Neisseria gonorrhoeae* □ *Neisseria gonorrhoeae*—synthesis of  $\omega$ -(4-aminophenylsulfonamido)alkyl disulfides and thiosulfates, activity against dihydropteroate synthetase, sulfanilamide-resistant □ Dihydropteroate synthetase—synthesis of  $\omega$ -(4-aminophenylsulfonamido)alkyl disulfides and thiosulfates, sulfanilamide-resistant *Neisseria gonorrhoeae*

The development of bacterial resistance to many of the antibiotics and synthetic antibacterials has reopened the search for synthetic antibacterials, particularly for use against resistant strains of bacteria. The inclusion of a reactive function in the sulfonamide molecule that might lead to an irreversible inhibitor of dihydropteroate synthetase appeared to offer a plausible approach to an agent of this type. The active site of dihydropteroate synthetase is not well defined, but it may be considered, like most enzymes, to contain disulfide bonds between juxtaposed cysteine residues. This provides an accessory receptor site which should bind with functional groups reactive toward disulfides. Accordingly, a series of *N*-sulfonamidoalkyl disulfides and thiosulfates (I) has been synthesized for testing against a drug-resistant organism. A sulfanilamide-resistant strain of *Neisseria gonorrhoeae* was available for this purpose (1). It has been shown (2) that carboxyl groups in the side chains of  $N_1$ -substituted sulfanilamides showed relatively high affinities toward a dihydropteroate synthesizing system of *Escherichia coli*, probably by interacting with an accessory area of the enzyme.

It is possible that a sulfonamido disulfide, previously



prepared in this laboratory, may have reacted with such an accessory receptor site. Bis[4-(4-acetamidobenzenesulfonamido)phenyl] disulfide was described as curative in mice infected with *Plasmodium berghei* (3). It was considered at the time that this molecule might be acting as a binding agent to DNA, but other related nonsulfonamido containing disulfides with good DNA binding potential showed no antimalarial activity (4).

Previously, other examples of molecules containing both sulfonamide and disulfide functions have been reported: several 4-aminobenzenesulfonamidoaryl disulfides (5) and two 4-aminobenzenesulfonamidoalkyl disulfides, where the alkyl function had two and three methylene units (6). Both series showed some antibacterial properties. No 4-aminobenzenesulfonamidoalkane thiosulfates have been reported as antibacterials.

## RESULTS AND DISCUSSION

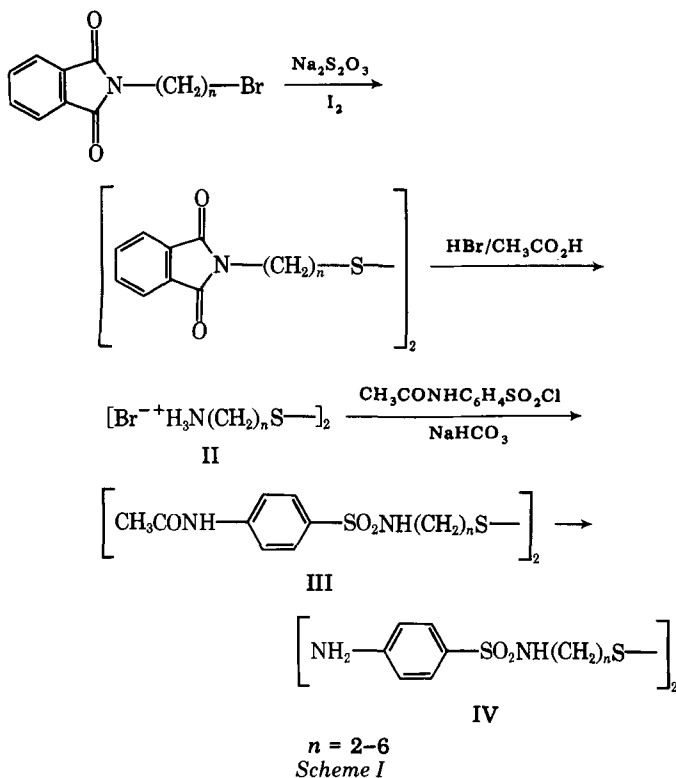
**Chemistry**—The synthesis of the 4-aminobenzenesulfonamidoalkyl disulfides is outlined in Scheme I. Reaction of polymethylene dibromides with potassium phthalimide gave the known  $\omega$ -bromalkylphthalimides, which were separated from byproduct diphtalimides by recrystallization from hexane. Treatment of the bromoalkylphthalimides with sodium thiosulfate gave intermediate thiosulfates which were oxidized *in situ* with iodine to give the disulfides. Although the literature recommends addition of solid iodine (7) for this conversion, a solution of the stoichiometric amount of iodine in methanol was found superior for these compounds.

Removal of the phthaloyl groups by hydrazinolysis failed, but the use of hydrobromic acid, mixed with acetic (8) or propionic acid was successful in giving the aminoalkyl disulfide hydrobromides (II).

Sulfonamide formation from *N*-acetylsulfanil chloride and amines generally is catalyzed with aqueous sodium hydroxide or carbonate or anhydrous pyridine. Because of the alkali-sensitive disulfide function, these catalysts were unsuitable; the use of sodium bicarbonate gave the desired products. All of the acetamidobenzenesulfonamidoalkyl disulfides (III) were difficult to purify; washing with an aqueous solution of ethanol and acetic acid generally provided good yields of reasonably pure material.

Deacetylation was carried out in a boiling mixture of hydrochloric acid–water–glycol (1:1:2) for 10 min; prolonged heating cleaved the sulfonamide as well. Since the dihydrochlorides of the higher homologs ( $n = 4-6$ ) were insoluble in hot water, the free amines (IV) were isolated by the action of ammonia on dilute solutions of the dihydrochlorides in hot methanol. With the  $n = 3$  homolog, the free base actually separated from the acid reaction mixture. All of the disulfides had similar IR spectra showing N—H stretching frequencies at 3345–3440 and 3255–3370  $\text{cm}^{-1}$ , and S—O stretching at 1322–1406 and 1138–1152  $\text{cm}^{-1}$ .

The preparation of the 4-aminobenzenesulfonamidoalkane thiosulfates is outlined in Scheme II. Although the *N*-acetyl derivative of the sulfonamidoethane thiosulfate is known (9), only free amino sulfonamides have shown antifolate activity. Since either acid or basic hydrolysis of the acetyls would decompose the thiosulfate function, the free amino thiosulfates were prepared from the 4-aminobenzenesulfonamidoalkyl



bromides on reaction with sodium thiosulfate. The intermediate amine hydrochloride, where  $n = 3$ , was surprisingly insoluble in water. Conversion to the free base gave a water-soluble sodium salt of the desired thiosulfate.

Since the disulfides proved in general to be too insoluble for enzyme inhibition tests, a carboxyl group was introduced for water solubilization by preparing the bis(4-aminobenzenesulfonamido) derivative (V) of L-cystine (10). The melting point of the product (192–194°) agreed with that reported previously (193–194°) (10).

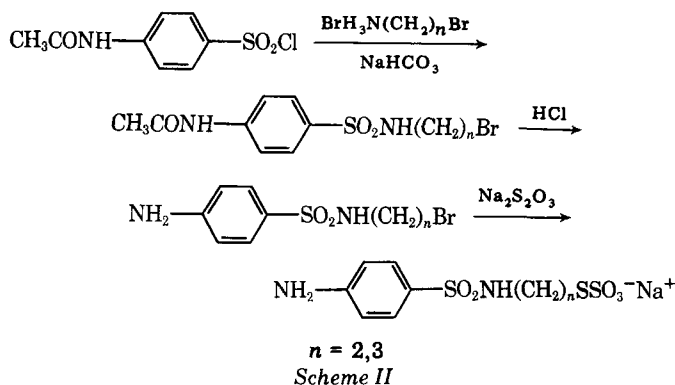
**Inhibition of Dihydropteroate Synthetase**—Determination of  $ID_{50}$  concentrations was carried out for sulfanilamide, sodium *p*-aminobenzenesulfonamidoethane (and -propane) thiosulfate, *N,N'*-bis(sulfanilyl)-L-cystine and *p*-aminobenzenesulfonamidopropyl bromide. The enzyme preparation used was an extract of a sulfanilamide-resistant strain of *N. gonorrhoeae* (1). Results are recorded in Table I.

Results of the inhibition study show that sulfanilamide itself showed the greatest inhibitory activity, although the  $ID_{50}$  values for the synthesized compounds fall within the same order of activity. It is possible that the additional bulk of the alkyl functions on the sulfonamide nitrogen reduces the binding ability of the compounds. If this were the case, it would be expected that the disulfide, with a nonlinear carboxyl group, would show the least inhibition. If the inhibition depended on a greater degree of hydrophobicity, then the bromoethyl derivative should have shown a greater inhibitory effect. It is apparent from the results, however, that a disulfide link with enzyme mercapto did not take place.

Since there was very little difference in inhibitory properties between the homologous thiosulfates, and the more hydrophobic bromoethyl derivative had even less activity, it is apparent that additional hydrophobic functions play no significant role in the binding at the receptor site. This finding agrees with a previous report (11) where it was found that inhibition of this enzyme by *N*<sub>1</sub>-phenylsulfonamides was not improved by greater hydrophobicity.

**Table I—Inhibition of Dihydropteroate Synthetase**

| Compound                                           | Concentration range, mM | $ID_{50}$ , $\mu M$ |
|----------------------------------------------------|-------------------------|---------------------|
| Sulfanilamide                                      | 0–0.6                   | 59                  |
| Sodium 4-aminobenzenesulfonamidoethanethiosulfate  | 0–1.2                   | 106                 |
| Sodium 4-aminobenzenesulfonamidopropanethiosulfate | 0–1.2                   | 108                 |
| <i>N,N'</i> -Bis(sulfanilyl)-L-cystine             | 0–1.2                   | 160                 |
| 4-Aminobenzenesulfonamidopropyl bromide            | 0–1.2                   | 250                 |



## EXPERIMENTAL<sup>1</sup>

**$\alpha$ -Bromoalkyl- $\omega$ -phthalimides**—These compounds were prepared essentially by the method of Klayman *et al.* (12). The crude products were recrystallized from ethyl alcohol, dried, recrystallized from hexane, and cooled at 25° for several hours and then cooled at 3°. Yields of 48–66% were obtained from material which melted at literature values (12).

**Bis( $\omega$ -Phthalimidoalkyl) Disulfides**—The procedure is a modification of that of Dirscherl and Weingarten (7). To a heated solution of water (75 ml), methanol (75 ml), and  $\alpha$ -bromoalkyl- $\omega$ -phthalimide (0.08 mole) was added 0.08 mole of sodium thiosulfate pentahydrate with stirring. After 1 hr at reflux temperature, the mixture was treated with 10.15 g (0.04 mole) of iodine in 60 ml of methanol during 45 min, followed by 10 min of refluxing. The two-phase system was stirred and cooled; the resulting solid was ground in a mortar, slurried with 150 ml of water for 1 hr, filtered, and dried. Yields of 89–95% were obtained from material whose melting points agreed with literature values (7, 8).

**$\omega,\omega'$ -Dithioalkanamine Dihydrobromides (II)**—A mixture of 0.04 mole of the appropriate bis( $\omega$ -phthalimidoalkyl) disulfide, 22 ml of 48% hydrobromic acid, and either 22 ml of acetic acid (for  $n = 3, 4$ ) or 22 ml of propionic acid (for  $n = 5, 6$ ) was refluxed for 24 hr. The hot orange liquid was poured into 50 ml of water and kept overnight at 25°. Phthalic acid was removed by filtration, and the filtrates were freed from acids by rotary evaporation. The residue was slurried with 40 ml of water and 0.3 g of activated charcoal and filtered, and the filtrate was slurried with 25 ml of water and evaporated to dryness. The residue was dissolved in 50–100 ml of hot ethyl alcohol, cooled to 25°, and treated with 50–200 ml of ether. The isolated solid was washed with ether and dried. Physical constants of products are listed in Table II.

**$\omega$ -(4-Acetamidophenylsulfonamido)alkyl Disulfides (III)**—Purified *N*-acetylsulfanilyl chloride (mp 144–149°) (17.53 g, 0.075 mole) was dissolved in 150 ml of acetone, and a solution of 0.035 mole of the appropriate  $\omega,\omega'$ -dithioalkanamine dihydrobromide in 50 ml of water was added with ice cooling the mixture. A solution of 13.45 g (0.16 mole) of sodium bicarbonate in 170 ml of water was added dropwise with stirring below 10° during 40 min. The resulting mixture was heated at 50° for 20 hr, cooled in an ice bath, filtered, and washed with water. The solid was slurried with a solution of 200 ml of water, 100 ml of ethyl alcohol, and 10 ml of acetic acid for 4 hr and filtered. The product was dried over sodium hydroxide at 25°/0.4–1.0 torr for 16–24 hr. Physical constants of products are listed in Table II.

**$\omega$ -(4-Aminophenylsulfonamido)alkyl Disulfides (IV)**—With magnetic stirring, 0.004 mole of the preceding diacetyl derivative, 5 ml of HCl, 5 ml of water, and 10 ml of ethylene glycol were refluxed for 10 min. A thick precipitate of the dihydrochloride was generally obtained, 40 ml of water was added, and the solid was filtered and dried at 25°/0.5–1.5 T for 18 hr over sodium hydroxide. The solid was dissolved in 250 ml of boiling methanol, treated with 0.3 g of activated carbon, and diluted with 10 ml of 4 *N* ammonium hydroxide and 200 ml of water to precipitate the free base. It was filtered and dried as before. Physical constants of products are listed in Table II.

<sup>1</sup> Melting points were determined in capillaries with a Mel-Temp block and needed no correction. IR spectra were taken in KBr pellets using a Perkin-Elmer 457 grating spectrophotometer. Elemental analyses were done by Dr. F. B. Strauss, Oxford, England or by Instranal Laboratory, Rensselaer, N.Y. TLC was carried out using silica gel plates and exposure to iodine vapor. Organic reagents were supplied by Aldrich Chemical Co. or by Eastman Organic Chemicals. [7-<sup>14</sup>C]*p*-Aminobenzoic acid (>95% radiochemical purity) was obtained from New England Nuclear Corp., Boston, Mass. Measurement of enzyme inhibition was done with a Packard Liquid Scintillation Spectrometer, model 3320. Frozen cells of sulfanilamide-resistant *Neisseria gonorrhoeae* strain 7134 were supplied by Johnna Ho, Harvard University School of Public Health.

Table II—Physical Constants of the Disulfides

| Compound | n | Melting point            | Yield, % | Formula                                                                      | Analysis, % |       |
|----------|---|--------------------------|----------|------------------------------------------------------------------------------|-------------|-------|
|          |   |                          |          |                                                                              | Calculated  | Found |
| II       | 3 | 230–232 <sup>a</sup>     | 86       |                                                                              |             |       |
| II       | 4 | 248–249 <sup>b</sup>     | 87       |                                                                              |             |       |
| II       | 5 | 256–258 dec.             | 82       | C <sub>10</sub> H <sub>24</sub> N <sub>2</sub> S <sub>2</sub> ·2HBr          | Br 40.13    | 39.80 |
| II       | 6 | 243–245 dec.             | 78       | C <sub>12</sub> H <sub>28</sub> N <sub>2</sub> S <sub>2</sub> ·2HBr          | Br 37.49    | 37.39 |
| III      | 2 | 190–192 <sup>c,d</sup>   | 92       |                                                                              |             |       |
| III      | 3 | 154–158 dec <sup>e</sup> | 77       |                                                                              |             |       |
| III      | 4 | 145–149                  | 68       | C <sub>24</sub> H <sub>34</sub> N <sub>4</sub> O <sub>6</sub> S <sub>4</sub> | C 47.81     | 47.95 |
|          |   |                          |          |                                                                              | H 5.68      | 5.68  |
|          |   |                          |          |                                                                              | N 9.30      | 9.21  |
| III      | 5 | 130–131                  | 81       | C <sub>26</sub> H <sub>38</sub> N <sub>4</sub> O <sub>6</sub> S <sub>4</sub> | C 49.50     | 49.48 |
|          |   |                          |          |                                                                              | H 6.07      | 5.89  |
|          |   |                          |          |                                                                              | N 8.88      | 8.30  |
| III      | 6 | 123–125                  | 72       | C <sub>28</sub> H <sub>42</sub> N <sub>4</sub> O <sub>6</sub> S <sub>4</sub> | C 51.03     | 51.13 |
|          |   |                          |          |                                                                              | H 6.43      | 6.28  |
|          |   |                          |          |                                                                              | N 8.50      | 8.47  |
| IV       | 2 | 130–131 <sup>f</sup>     | 92       |                                                                              |             |       |
| IV       | 3 | 152–153 <sup>g</sup>     | 96       |                                                                              |             |       |
| IV       | 4 | 176–177                  | 91       | C <sub>20</sub> H <sub>30</sub> N <sub>4</sub> O <sub>4</sub> S <sub>4</sub> | C 46.31     | 46.45 |
|          |   |                          |          |                                                                              | H 5.83      | 5.71  |
|          |   |                          |          |                                                                              | N 10.80     | 10.51 |
| IV       | 5 | 166–167                  | 87       | C <sub>22</sub> H <sub>34</sub> N <sub>4</sub> O <sub>4</sub> S <sub>4</sub> | C 48.32     | 48.50 |
|          |   |                          |          |                                                                              | H 6.27      | 6.09  |
|          |   |                          |          |                                                                              | N 10.25     | 10.20 |
| IV       | 6 | 164–165.5                |          | C <sub>24</sub> H <sub>38</sub> N <sub>4</sub> O <sub>4</sub> S <sub>4</sub> | C 50.14     | 50.59 |
|          |   |                          |          |                                                                              | H 6.66      | 6.42  |
|          |   |                          |          |                                                                              | N 9.75      | 9.79  |
|          |   |                          |          |                                                                              | S 22.31     | 22.30 |
| V        |   | 192–194 <sup>h</sup>     | 62       | C <sub>18</sub> H <sub>22</sub> N <sub>4</sub> O <sub>8</sub> S <sub>4</sub> |             |       |

<sup>a</sup> Lit. (6) mp 232°. <sup>b</sup> Lit. (7) mp 240°. <sup>c</sup> Commercially available 2,2'-dithiobisethanamine dihydrochloride was used as reagent instead of the hydrobromide. <sup>d</sup> Lit. (6) mp 194–196.5°. <sup>e</sup> Lit. (6) mp 162–163°. <sup>f</sup> Lit. (6) mp 129°. <sup>g</sup> Lit. (6) mp 154.5–155.5°. <sup>h</sup> Lit. (10) mp 193–194°.

**2-(4-Acetamidophenylsulfonamido)ethyl Bromide**—This was prepared from *N*-acetylsulfanilyl chloride and 2-bromoethylamine hydrobromide<sup>2</sup> by the same general procedure used for the  $\omega$ -(4-acetamidophenylsulfonamido)alkyl disulfides. The crude product was slurried with aqueous sodium bicarbonate, filtered, and dried to give a 77% yield of material melting at 168–169° [lit. (13) mp 168–169°].

**2-(4-Aminophenylsulfonamido)ethyl Bromide**—The previous compound (9.63 g, 0.03 mole), 10 ml of 38% HCl, and 10 ml of 95% ethyl alcohol were refluxed for 30 min and diluted with 125 ml of water. The solution was decolorized with activated carbon and neutralized with sodium bicarbonate; the free base was filtered, slurried with 100 ml of water, filtered, and dried at 25°/0.8 torr for 20 hr to give an 84% yield of tan solid, mp 87.5–88.5° [lit. (14) mp 90–91°].

**Sodium 2-(4-Aminophenylsulfonamido)ethanethiosulfate**—A mixture of 5.97 g (0.024 mole) of sodium thiosulfate pentahydrate, 6.72 g (0.024 mole) of the previous product, 25 ml of methanol, and 25 ml of water was refluxed for 30 min and cooled to 25°. Solvent was removed by rotary evaporation, and the resulting solid was dried further by evaporation of 2 × 25 ml of absolute ethanol. The residue was shaken with 15 ml of dimethylformamide, cooled overnight, and the insoluble sodium bromide was removed. The filtrate was diluted with 50 ml of acetone, filtered after several hours, and the residue was dried at 20°/0.3 torr for 16 hr to give 8.81 g of crude solid, mp 178–182°. Recrystallization from 95% ethyl alcohol at –20° gave a 44% yield of colorless solid, mp 207.5–209°(dec), which gave a negative test for halogen.

*Anal.*—Calc. for C<sub>8</sub>H<sub>11</sub>N<sub>2</sub>NaO<sub>5</sub>S<sub>3</sub>: C, 28.73; H, 3.32; N, 8.38; S, 28.77. Found: C, 28.76; H, 3.17; N, 8.39; S, 28.39.

**3-(4-Acetamidophenylsulfonamido)propyl Bromide**—This compound was prepared by the same method used for 2-(4-acetamidophenylsulfonamido)ethyl bromide, from *N*-acetylsulfanilyl chloride and 3-bromopropylamine (8). After heating, the solution was decolorized with activated carbon, diluted with 200 ml of water, and cooled to 3° before filtration to give a crude product that was recrystallized from 50% ethyl alcohol to give a 64% yield of tan prisms, mp 104–105.5°.

*Anal.*—Calc. for C<sub>11</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>3</sub>S: C, 39.41; H, 4.51; Br, 23.84. Found: C, 39.61; H, 4.20; Br, 24.10.

**3-(4-Aminophenylsulfonamido)propyl Bromide**—The previous compound (16.00 g, 0.048 mole), 20 ml of 38% HCl, and 20 ml of 95% ethyl alcohol were refluxed for 30 min, and the solid mass was transferred to a beaker with 100 ml each of water and 95% ethyl alcohol. After being heated, the solution was decolorized with activated carbon, cooled to 25°, and neutralized with sodium bicarbonate. The free base was filtered,

slurried with 100 ml of water, and dried at 23°/0.2 torr for 16 hr to give 9.45 g (68%) of shiny, white prisms, mp 106–106.2°.

*Anal.*—Calc. for C<sub>9</sub>H<sub>13</sub>BrN<sub>2</sub>O<sub>2</sub>S: C, 36.86; H, 4.47; Br, 27.26; S, 10.94. Found: C, 37.05; H, 4.43; Br, 27.54; S, 10.75.

**Sodium 3-(4-Aminophenylsulfonamido)propanethiosulfate**—A mixture of 1.29 g (0.0052 mole) of sodium thiosulfate pentahydrate, 1.52 g (0.0052 mole) of the previous product, 5 ml of methanol, and 5 ml of water was refluxed for 25 min, cooled, and freed of solvent by rotary evaporation. After further evaporation with 5 ml of absolute ethanol, the remaining gum was leached with 20 ml of 97.5% ethyl alcohol and cooled overnight. The solid sodium bromide was removed, and the filtrate was diluted with 30 ml of 1-propanol and cooled to –20° to give 0.9 g of white solid, which was recrystallized from 18 ml of 95% ethyl alcohol at –20° to give 0.6 g (33%) of white prisms, mp 212.5–213.5°.

*Anal.*—Calc. for C<sub>9</sub>H<sub>13</sub>N<sub>2</sub>NaO<sub>5</sub>S<sub>3</sub>: C, 31.02; H, 3.78; N, 8.04. Found: C, 31.29; H, 3.64; N, 8.05.

**Determination of Enzyme Inhibition**—ID<sub>50</sub> values for the inhibition of dihydropterolate synthesis using a cell-free extract of dihydropterolate synthetase from *N. gonorrhoeae* (1) were determined for the test compounds using six inhibitor concentrations in duplicate experiments. The ID<sub>50</sub> value was taken from the plot of the percent inhibition against the logarithm of the inhibitor concentration.

Solutions of the test compounds were made to contain the following components in a 200- $\mu$ l volume: tris(hydroxymethyl)aminomethane hydrochloric acid buffer (140 mM, pH 5.8), dihydropteridine pyrophosphate (7  $\mu$ M) (15), [7-<sup>14</sup>C]p-aminobenzoic acid (15  $\mu$ M), magnesium chloride hexahydrate (5 mM), 2-mercaptoethanol (0.1 M), test compound (0–1.2 mM), and enzyme (0.14 mg of protein). The reaction mixture without dihydropteridine pyrophosphate served as blank. After the mixture was incubated at 37° for 20 min, the reaction was stopped by the addition of 2-mercaptoethanol (20  $\mu$ l). The amount of labeled dihydropterolate was measured as follows.

A portion (100  $\mu$ l) of the reaction mixture was spotted on 3-mm chromatographic paper<sup>3</sup> (22.86 × 29.21 cm). The chromatogram was developed in descending fashion by immersing the lower margin of the paper in potassium phosphate buffer (0.1 M, pH 7.0) inside a chamber at room temperature. After the solvent front had moved 15 cm from the origin, the paper was removed and dried. Neither the labeled dihydropterolate nor the labeled pterolate moved under these conditions, whereas unreacted [7-<sup>14</sup>C]p-aminobenzoic acid migrated with an *R<sub>f</sub>* of 0.78. An area (3 × 3 cm) of the paper around the origin was cut into small pieces and put into a counting vial containing 10 ml of scintillation fluid consisting

<sup>2</sup> Aldrich Chemical Co.

<sup>3</sup> Whatman.

of the following in a volume of 1 liter: naphthalene (150 g), 2,5-diphenyl-oxazole (8 g), 1,4-bis(4-methyl-5-phenyl-oxazol-2-yl)benzene (0.6 g), ethylene glycol (20 ml), 2-ethoxyethanol (100 ml), and toluene to make 1 liter. The radioactivity was measured in the liquid scintillation counter with an 18% gain setting. Each vial was counted twice, and the data represent the average of two counts. Results are shown in Table I.

## REFERENCES

- (1) R. I. Ho, L. Corman, S. A. Morse, and M. S. Artenstein, *Antimicrob. Agents Chemother.*, **5**, 388 (1974).
- (2) H. H. W. Thijssen, *J. Med. Chem.*, **20**, 233 (1977).
- (3) W. O. Foye and J. P. Speranza, *J. Pharm. Sci.*, **59**, 259 (1970).
- (4) W. O. Foye, J. J. Lanzillo, Y. H. Lowe, and J. M. Kauffman, *ibid.*, **64**, 211 (1975).
- (5) S. Minakami and M. Kono, Japan Pat. 2425 (1967).
- (6) R. Lehmann and E. Grivsky, *Bull. Soc. Chim. Belg.*, **55**, 52 (1946).
- (7) W. Dirscherl and F. W. Weingarten, *Justus Liebigs Ann. Chem.*, **574**, 131 (1951).
- (8) A. Schöberl, M. Kawohl, and G. Hansen, *ibid.*, **614**, 83 (1958).

- (9) W. O. Foye, Y.-L. Lai-Chen, and B. R. Patel, *J. Pharm. Sci.*, **70**, 49 (1981).
- (10) F. Irreverre and M. X. Sullivan, *J. Am. Chem. Soc.*, **64**, 1488 (1942).
- (11) G. H. Miller, P. H. Doukas, and J. K. Seydel, *J. Med. Chem.*, **15**, 700 (1972).
- (12) D. L. Klayman, M. M. Grenan, and D. P. Jacobus, *ibid.*, **12**, 510 (1967).
- (13) A. A. Goldberg and W. Kelly, *J. Chem. Soc.*, **1948**, 1919.
- (14) E. L. Jackson, *J. Am. Chem. Soc.*, **72**, 395 (1950).
- (15) R. I. Ho, L. Corman, and W. O. Foye, *J. Pharm. Sci.*, **63**, 1474 (1974).

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# Optical Purity Determination by NMR: Use of Chiral Lanthanide Shift Reagents and a Base Line Technique

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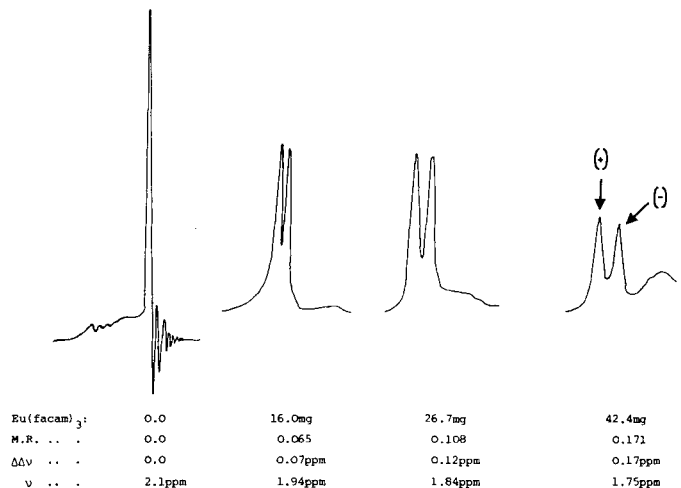
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**Abstract** □ A method for optical purity determination of a range of chiral drug molecules by NMR spectroscopy is reported. This technique involves the use of optically active lanthanide shift reagents and a newly developed base line analysis. Its applicability was demonstrated for a variety of drugs including nonsteroidal antiinflammatory agents and some adrenergic agents. It is established that successful application of the method depends on a constant shift reagent to sample molar ratio, constant instrumental conditions for all solutions, and the use of a calibration curve derived from solutions containing the same total concentration of the two enantiomers. For the examples cited, the correlation coefficient is not <0.97, and a mathematical treatment is included which supports the basis of the method.

**Keyphrases** □ Optical purity determination—by NMR spectroscopy, use of chiral lanthanide shift reagents and a base line technique □ Chiral lanthanide shift reagents—determination of optical purity by NMR spectroscopy, base line technique □ NMR spectroscopy—determination of optical purity, use of chiral lanthanide shift reagents and a base line technique

The application of NMR spectroscopy to the quantitative analysis of pharmaceuticals has become widespread (1, 2) since the publication of Hollis (3) on the determination of aspirin, phenacetin, and caffeine mixtures. Provided that careful consideration is given to solvent, internal standard, and instrumental conditions (including spinning sidebands and carbon 13 satellites), mixtures, often of some complexity, can be analyzed with a high degree of accuracy. Optical purity determination is another aspect of the analysis of any drug presented in a resolved form (enantiomer) or the racemate of a particular diastereoisomer. Apart from polarimetry, which has been ex-

tensively employed despite its drawbacks, optical purity determination has been achieved by chromatographic methods such as GLC (4, 5) and HPLC (6, 7), isotope dilution (8), kinetic resolution (9), and NMR. Even before the discovery of chiral lanthanide shift reagents, the NMR method was used for optical purity measurement either by diastereoisomer formation (10, 11) or the application of chiral solvents (12, 13). Since the publication of Whitesides and Lewis (14) on the relatively large frequency differences between corresponding resonances of enan-



**Figure 1**—Resonance of N—CH<sub>3</sub> of (±)-ephedrine in deuterated benzene on incremental addition of III.