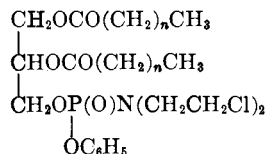


TABLE I  
1,2-Diglycerides 3-[Hydrogen bis(2-chloroethyl)phosphoramidate phenyl ester]



Compd	$n^a$	Empirical formula	Yield, %	Mp, °C	$n_{20}^D$	$R_f^b$	Analyses <sup>c</sup>
3a	16	C <sub>49</sub> H <sub>88</sub> Cl <sub>2</sub> NO <sub>7</sub> P	58	29–32		0.33	C, H, N
3b		C <sub>49</sub> H <sub>84</sub> Cl <sub>2</sub> NO <sub>7</sub> P	30		1.4807	0.30	C, H, N
3c	14	C <sub>45</sub> H <sub>80</sub> Cl <sub>2</sub> NO <sub>7</sub> P	53	37–39		0.33	C, H, N
3d	12	C <sub>41</sub> H <sub>72</sub> Cl <sub>2</sub> NO <sub>7</sub> P	60	39–40		0.30	C, H, N
3e	10	C <sub>37</sub> H <sub>64</sub> Cl <sub>2</sub> NO <sub>7</sub> P	59		1.4802	0.30	C, H
3f	8	C <sub>33</sub> H <sub>56</sub> Cl <sub>2</sub> NO <sub>7</sub> P	59		1.4803	0.27	C, H, N
3g	7	C <sub>31</sub> H <sub>52</sub> Cl <sub>2</sub> NO <sub>7</sub> P	54		1.4838	0.27	C, H, N
3h	6	C <sub>29</sub> H <sub>48</sub> Cl <sub>2</sub> NO <sub>7</sub> P	25		1.4800	0.26	C, H, N

<sup>a</sup> 3b = 1,2-disubstituted unsat olein [hydrogen bis(2-chloroethyl)phosphoramidate Ph ester]. Silica,<sup>5</sup> hexane-Et<sub>2</sub>O (60:40).  
<sup>c</sup> See ref 5.

no significant prolongation of survival time. These results strongly indicate that lack of activity is this tumor of the "cytotoxic" N mustard moiety of these analogs is not related to significant differences in lipid solubilities or other physicochemical properties, and broadly confirm literature reports<sup>3,4</sup> that antineoplastic activity in phosphoramidate and phosphoesteramidic mustards is greatly diminished or abolished by substituents such as Ph.

#### Experimental Section<sup>5</sup>

*N,N*-Bis(2-chloroethyl)phosphoramidic acid (2,2-dimethyl-1,3-dioxolanyl-4-yl)methyl phenyl ester (1) and *N,N*-bis(2-chloroethyl)phosphoramidic acid 2,3-dihydroxypropyl phenyl ester (2) were prepd by the method of Batrakov, *et al.*;<sup>1</sup> upon prepn 2 was used directly for acylation reactions. Deriv 3b was prepd according to the procedure described for the synthesis of 3a–3h except that acylation was carried out at 70°. Spectral data (ir, pmr) were nearly identical for each 3 homolog and are reported only for 3c. Some physical properties of 3a–3h are included in Table I.

**1,2-Diglycerides 3-[Hydrogen bis(2-chloroethyl)phosphoramidate, phenyl ester] (3a–3h).**—To 1.48 g (0.004 mole) of 2 in 8 ml of pyridine (0°) was added 3.3 g (0.012 mole) of palmitoyl chloride, and the mixt was kept stirring at room temp for 48 hr. The reaction mixt then was poured into ice H<sub>2</sub>O and the solid material was extd with three 25-ml portions of Et<sub>2</sub>O. The exts were combined and washed successively with H<sub>2</sub>O, ice-cold 2% H<sub>2</sub>SO<sub>4</sub>, and again with H<sub>2</sub>O. The soln was dried (MgSO<sub>4</sub>), filtered, and concd under reduced pressure to give 4 g of a crude yellow oil, which was placed on an Al<sub>2</sub>O<sub>3</sub> (100 g) column (60 × 2 cm) and was eluted with 600 ml of CHCl<sub>3</sub>. Concn of the eluate gave 2.6 g of a yellow viscous liq which showed a major ( $R_f$  0.33) and a minor ( $R_f$  0.6) spot on tlc (silica gel, hexane–Et<sub>2</sub>O, 60:40). The viscous material then was chromatogd on a silica gel (50 g) column (60 × 2 cm) with hexane–Et<sub>2</sub>O (60:40) as eluent (450

ml). Fractions which showed one spot ( $R_f$  0.33) were combined and concd under reduced pressure to obtain 1.8 g (53%) of a colorless oil. The oil was dissolved in 15 ml of petr ether (bp 30–80°) and kept at –10° for 8 hr, during which time an amorphous, white solid sepd. Recrystn using Norite A (boiling, 5 min) gave 3c: mp 37–39° (lit.<sup>1</sup> 35.5–37°);  $R_f$  0.33, silica gel, hexane–ether (60:40); ir (Nujol) 1748 (C=O), 1262 (P=O), 1200 (POC<sub>arom</sub>) 1090 (PN), 1060 (POC<sub>aliph</sub>), 762 cm<sup>-1</sup> (CCl<sub>4</sub>); 100-MHz pmr (CDCl<sub>3</sub>)  $\tau$  2.64–2.85 (m, 5), 4.73 (t, 1), 5.52–5.98 (m, 4) 6.33–6.88 (m, 8), 7.72 (t, 4), 8.75 (s, 52) 9.14 (t, 6).

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#### New Thio Derivatives of Carcinogenic Arylamines.

##### 5. Ring-Substituted

##### Methylthio-4-acetamidostilbenes<sup>1a</sup>

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In earlier papers in this series we described the synthesis of some new thiofluorenes<sup>1b–d</sup> related to the metabolism of the carcinogen 2-acetamidofluorene, and of a similar new thio derivative of 4-acetamidodiphenyl.<sup>1e</sup>

The carcinogen *N*-hydroxy-4-acetamidostilbene [*N*-(OH)-4-AAS] yielded a more complex metabolic pic-

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(4) O. M. Friedman, *ibid.*, **51**, 347 (1967).

(5) Fatty acyl chlorides were obtained from Eastman Kodak Co., Rochester, N. Y. 14650, and Sigma Chemical Co., St. Louis, Mo. 63118, and were used without further purification. Silicar tlc 7GF (Mallinckrodt) was used for tlc analyses; column chromatog purifications were made with aluminum oxide, neutral, and silica gel (E. Merck AG). The ir spectra (neat, Nujol) were detd with Beckman IR-8 and Perkin-Elmer 521 spectrophotometers, and pmr spectra with Varian A-60 and HA-100 spectrometers using CDCl<sub>3</sub> (Me<sub>4</sub>Si) as solvent. Melting points were detd with a Thomas-Hoover capillary melting point apparatus and are uncor. Refractive indices were detd with a Bausch and Lomb Abbe-3L refractometer. Where analyses (Table I) are indicated only by the symbols of the elements, anal. results obtained for those elements were within ±0.4% of the theor values. Analyses were performed by Micro-Analysis, Inc., Marshallton, Wilmington, Del.

(1) (a) Supported in part by a grant (CA-01744) from the National Cancer Institute, National Institutes of Health, and in part by Research Career Development Award 5-K3-CA-14,991 (T.L.F.); (b) T. L. Fletcher, M. J. Namkung, and H.-L. Pan, *J. Med. Chem.*, **10**, 936 (1967); (c) M. J. Namkung and T. L. Fletcher, *ibid.*, **11**, 1235 (1968); (d) H.-L. Pan, M. J. Namkung, and T. L. Fletcher, *ibid.*, **11**, 1236 (1968); (e) T. L. Fletcher, C.-A. Cole, H.-L. Pan, and M. J. Namkung, *ibid.*, **13**, 784 (1970).

ture.<sup>2</sup> The nonenzymatic reaction of *N*-acetoxy-4-AAS with methionine under physiological conditions yielded 4 products, each of which had a correct mol wt for  $\alpha$ -methylthio-4-AAS. Products with the same chromatographic and mass spectral properties as 3 of these *in vitro* products were also liberated from the liver protein of the rats administered *N*(OH)-4-AAS. We, therefore, undertook the synthesis of all 5 of the ring methylthio substituted derivatives of 4-AAS, in order to aid in structure confirmation of these compounds.

Both 2- and 3-methylthio-4-nitrostilbene were made by condensing the appropriate methylthio-4-nitrotoluene with PhCHO, using a method reported by Farrissey, *et al.*<sup>3</sup> The intermediate aldol formed in the first of these was dehydrated in refluxing DMSO. The corresponding 2'- and 4'-methylthio isomers were made similarly, starting with *p*-nitrotoluene and *o*- or *p*-methylthiobenzaldehyde. In each of these cases there was intermediate aldol formation; dehydration to the stilbene was accomplished in boiling DMSO. Initial attempts to effect dehydration of the 2 and 2' isomers in DMSO failed, because the reaction time was too short.<sup>4</sup> However, refluxing for 18–24 hr followed by boiling down to small vol gave excellent yields of the desired stilbenes.

The fifth of these isomers, *i.e.*, 3'-methylthio-4-nitrostilbene, was synthesized by a Meerwein arylation reaction between 3-methylthiobenzenediazonium chloride and *p*-nitrocinnamic acid. The product was recovered in low yield (by weight), but 90% yield based on recovered *p*-nitrocinnamic acid. Use of DMSO–Me<sub>2</sub>CO as the reaction solvent gave the best yields.

**Biological Results.**<sup>2</sup>—Comparison (glc, tlc, and mass spectrometry) of each of these 5 *S*-methyl-4-AAS derivatives with the products formed from *N*-acetoxy-4-AAS and methionine, or isolated from the liver proteins of rats administered *N*(OH)-4-AAS, shows an apparent identity of 3-methylthio-4-AAS with one of the *in vitro* and one of the *in vivo* products. One product formed both *in vitro* and *in vivo* shows no correspondence with any of the 5 synthetic methylthio derivatives. The other products have some similarity to light-induced forms (*cis*?) of 4'-methylthio-4-AAS and 2-methylthio-4-AAS, but identity has not been established.

Further work in this series is now being directed toward the  $\alpha$ - and  $\beta$ -methylthio-4-AAS derivatives.

#### Experimental Section<sup>5</sup>

**3-Methylthio-4-nitrotoluene (1).**—To a soln of 10.3 g (0.047 mole) of 3-bromo-4-nitrotoluene, mp 35–36° (lit.<sup>6</sup> 35–36°, from the AcO<sub>2</sub>H oxidn of 2-bromo-4-methylaniline)<sup>1b</sup> in 5 ml of DMSO, a soln of NaSMel<sup>1b</sup> in abs EtOH (0.05 mole; 36 ml) was added at room temp with swirling. In a few min the temp of the mixt

rose to 40°, and a yellow ppt came out. The ppt was filtered off, washed with EtOH, and dried, giving 9 g of product, mp 108–114°. One recrystn (C<sub>8</sub>H<sub>8</sub>) gave 6.1 g (70%) of product, mp 116–117°. *Anal.* (C<sub>8</sub>H<sub>8</sub>NO<sub>2</sub>S) N.

**3-Methylthio-4-nitrostilbene (2).**—To a soln of 15 g (0.082 mole) of 1 in 50 ml of DMF, 8.8 g (0.083 mole) of freshly purified PhCHO<sup>7</sup> and 0.1 g of freshly powd NaOH were added and the flask was corked. After 16 hr at room temp the mixt was dild with 125 ml of 5% HCl and stirred. The oily solid was filtered off and dissolved in 200 ml of EtOAc (Darco), filtered, boiled down to 100 ml, and the brown ppt then filtered off and washed with petr ether (bp 30–60°), giving 6.7 g (28%) of product, mp 124–125°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N, S.

**3-Methylthio-4-stilbenamine (3).**—A soln of 5.7 g of 2 in 150 ml of EtOH was boiled for 15 min with 10 ml of hydrazine hydrate (100%) and 0.1 g of Pd/C (5%), filtered, and boiled down to near dryness. Cooling and scratching with a glass rod gave 4.2 g (83%) of white ppt, mp 75–79°. One recrystn (MeOH) gave mp 78–79°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NS) C, H, N, S.

***N*-4-(3-Methylthiostilbenyl)acetamide (4).**—Acetylation of the above amine (Ac<sub>2</sub>O in C<sub>6</sub>H<sub>6</sub>) gave the amide, mp 151–152°. One recrystn (EtOH) gave a sample with the same mp. *Anal.* (C<sub>17</sub>H<sub>15</sub>NOS) C, H, N.

**1-Phenyl-2-(2-methylthio-4-nitrophenyl)ethanol (5).**—Condensn of 2-methylthio-4-nitrotoluene<sup>8</sup> with PhCHO, as described for 2, followed by recrystn of the crude product (C<sub>8</sub>H<sub>8</sub>, Darco) gave a 28% yield of 5, mp 133.5–134.5°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N; mol wt.<sup>9</sup>

**2-Methylthio-4-nitrostilbene (6).**—A soln of 2.9 g of 5 in DMSO (30 ml) was refluxed for 18 hr, boiled down to 15 ml, cooled, and poured into 50 ml of H<sub>2</sub>O to give 2.2 g (81%) of yellow ppt, mp 110–113°. One recrystn (EtOH, Darco) gave an anal. sample, mp 112–113°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N.

**2-Methylthio-4-stilbenamine·HCl (7).**—Hydrazine hydrate (100%) and Pd/C (5%) reduction of 6 (1.2 g) in the usual way (reflux 1.5 hr) gave the amine as an oil which was recovered as the HCl salt (0.85 g from EtOH), mp 174–176°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NS·HCl) N.

***N*-4-(2-Methylthiostilbenyl)acetamide (8).**—Compd 7 (0.85 g) was treated with dil NH<sub>4</sub>OH and the resulting oil was extd with Et<sub>2</sub>O, washed with H<sub>2</sub>O, dried, and evapd. The oil in 10 ml of C<sub>6</sub>H<sub>6</sub> was acetylated (Ac<sub>2</sub>O) to give 0.8 g, mp 157–158°. Recrystn (EtOH) gave the amide with unchanged mp. *Anal.* (C<sub>17</sub>H<sub>15</sub>NOS) C, H, N, S; mol wt.<sup>9</sup>

**Methyl 2-Methylthiobenzoate (9).**—To a soln of 19 g (0.475 mole) of NaOH in 400 ml of EtOH, 75 g (0.445 mole) of methyl 2-thiolbenzoate<sup>10</sup> was added with thorough stirring. A white ppt formed and (with the mixt at 35°) 65 g (0.445 mole) of MeI was added. An exothermic reaction (45°) resulted in complete soln of the ppt. The mixt was refluxed for 2 hr, reduced to 100 ml (white ppt), poured into 500 ml of hot (80°) H<sub>2</sub>O, and cooled giving 80 g (98%) of the product, mp 64–66° (lit.<sup>10</sup> mp 66–68°).

**2-Methylthiobenzohydrazide (10).**—A mixt of 80 g (0.44 mole) of 9, 185 ml of EtOH, and 90 ml of hydrazine hydrate (100%) was refluxed for 4 hr, boiled down to 100 ml, and cooled. Dildn with 100 ml of H<sub>2</sub>O gave 70 g (87%) of the hydrazide, mp 103–104° (lit.<sup>10</sup> mp 103–104°).

**1-(4-Toluenesulfonyl)-2-(2-methylthiobenzoyl)hydrazine (11)** was made by the lit. procedure<sup>11</sup> with a 90% yield, mp 142–143° (lit.<sup>11</sup> mp 132–133°).

**2-Methylthiobenzaldehyde (12)** was made essentially as reported,<sup>11</sup> from 91.5 g of 11, 33 g of Na<sub>2</sub>CO<sub>3</sub>, 600 ml of diethylene glycol, and 0.5 g of powd soft glass. The crude prod was distd giving 29.5 g (73%), bp 147–149° (16 mm), *n*<sub>D</sub><sup>20</sup> 1.6320 [lit.<sup>11</sup> bp 92° (0.09 mm), *n*<sub>D</sub><sup>20</sup> 1.6270].

**1-(2-Methylthiophenyl)-2-(4-nitrophenyl)ethanol (13).**—Condensn of 13.7 g of *p*-nitrotoluene in 100 ml of DMF, 15.2 g of 12, and 0.2 g of powd NaOH as described for 2, followed by recrystn from C<sub>6</sub>H<sub>6</sub> (Darco) gave 14.3 g (50%) of product, mp 122–123.5° with softening. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N; mol wt.<sup>9</sup>

(2) The biological information in this paper has been kindly supplied, before publication, by Drs. J. A. and E. C. Miller, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wis.

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(4) An alternate method, using 48% HBr in AcOH, gave us benzo(b)thiophene derivatives. This will be the subject of a separate report elsewhere.

(5) Boiling points are not corrected. Melting points were taken on a Fisher-Johns block and are corrected to standards. Where analyses are indicated only by symbols of the elements, anal. results were within  $\pm 0.4\%$  of the theor values. Analyses were performed by A. Bernhardt, Elbach über Engelskirchen, West-Germany.

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(9) The mol wt data on these compounds, determined by mass spectrometry, were kindly supplied by Dr. J. A. Miller, University of Wisconsin.

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In certain runs the mp was much wider indicating that some of the stilbene had already formed. Such mixts were as suitable to use in the following procedure as pure 13.

**2'-Methylthio-4-nitrostilbene (14).**—A soln of 5.8 g of 13 in DMSO (40 ml) was refluxed for 24 hr then boiled down to 10 ml, cooled, and poured into 50 ml of H<sub>2</sub>O to give 4.9 g (90%), mp 108–110°. One recrystn (EtOH, Darco) gave 4.3 g (80%) of the product, mp 109.5–110°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N.

**2'-Methylthio-4-stilbenamine (15).**—Hydrazine hydrate (100%) and Pd/C (5%) reduction of 1.5 g of 14 (1 hr) was followed by evapn to near dryness. Addn of petr ether (bp 30–60°) with stirring, gave a light brown ppt, 1.2 g (93%), mp 62–65°. One recrystn from EtOH–petr ether (bp 30–60°) gave an anal. sample, mp 65–66°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NS) N.

**N-4-(2'-Methylthiostilbenyl)acetamide (16).**—Acetylation gave the amide, mp 162–162.5°. *Anal.* (C<sub>17</sub>H<sub>17</sub>NOS) C, H, N, S; mol wt.<sup>9</sup>

**3'-Methylthio-4-nitrostilbene (17).**—3-Methylthioaniline [tech, Aldrich, redistd at 156.5–157° (16 mm)] (4.2 g, 0.03 mole) was mixed with HCl (7.8 ml), DMSO (2 ml), and H<sub>2</sub>O (15 ml). The mixt was stirred at 0–5° while a soln of NaNO<sub>2</sub> (2.1 g, 0.03 mole) in H<sub>2</sub>O (5 ml) was added dropwise in 15 min. After stirring at 0° for 30 min, the soln was added in one portion to a stirred mixt of *p*-nitrocinnamic acid (mp 287.5–290° dec) (5 g, 0.025 mole), DMSO (50 ml), and Me<sub>2</sub>CO (100 ml) at 0°. This was immediately followed by addn of anhyd NaOAc (8 g) and CuCl<sub>2</sub>·2H<sub>2</sub>O (5.1 g, 0.03 mole, in 8 ml of H<sub>2</sub>O) with continuous stirring at 0° for 2 hr, then at 25° for 18 hr. The mixt was then dild with H<sub>2</sub>O (350 ml). The solvent and some oily material were removed by steam distn. The residue was filtered off and extd with boiling C<sub>6</sub>H<sub>6</sub> (200 ml). The insol *p*-nitrocinnamic acid was recovered by alk extn (3.8 g). The C<sub>6</sub>H<sub>6</sub> ext was washed with 5% NaOH and H<sub>2</sub>O and dried (MgSO<sub>4</sub>). Evapn and column chromatog (Al<sub>2</sub>O<sub>3</sub>–C<sub>6</sub>H<sub>6</sub>) gave the product (1.8 g). Recrystn from EtOH gave orange crystals, 1.5 g (90%, based on the amt of *p*-nitrocinnamic acid consumed), mp 117–119°. One recrystn (EtOH) gave an anal. sample, mp 118–119°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N.

**3'-Methyl-4-stilbenamine (18).**—Hydrazine hydrate (100%) and Pd/C (5%) reduction of 17 gave the amine, mp 111–112° (EtOH–H<sub>2</sub>O). *Anal.* (C<sub>15</sub>H<sub>13</sub>NS) C, H.

**N-4-(3'-Methylthiostilbenyl)acetamide (19).**—Acetylation (Ac<sub>2</sub>O in AcOH) and recrystn (EtOH) gave the amide, mp 133–134°. *Anal.* (C<sub>17</sub>H<sub>17</sub>NOS) C, H, N, S; mol wt.<sup>9</sup>

**4-Methylthiobenzaldehyde (20).**—To a soln of KSM<sub>e</sub>, prepd by dissolving 26.4 g of powd KOH in 240 ml of EtOH and adding 20 g of MeSH at 0°, 56 g of *p*-chlorobenzaldehyde was added, and the mixt was refluxed for 3 hr, dild with 400 ml of H<sub>2</sub>O, and extd with CCl<sub>4</sub>. The org layer was sep'd and dried (Na<sub>2</sub>SO<sub>4</sub>), and the solvent distd off to give 52 g (85.5%) of the aldehyde, bp 163–165° (22 mm) [lit.<sup>12,13</sup> bp 99–100° (1.3 mm), 153° (17 mm)].

**1-(4-Methylthiophenyl)-2-(4-nitrophenyl)ethanol (21).**—Compd 20 (7.6 g, 0.05 mole) and *p*-nitrotoluene (6.85 g, 0.05 mole) were combined as described for 2. The mixt was poured into dil HCl and extd with C<sub>6</sub>H<sub>6</sub> (300 ml), and the ext was dried (Na<sub>2</sub>SO<sub>4</sub>). After evapn of the solvent, addn of 150 ml of cyclohexane gave 2.7 g (20%) of 4'-methylthio-4-nitrostilbene, mp 172–174°. Evapn of the filtrate to near dryness gave 6.4 g (44%) of 21, mp 121–122°. Recrystn (EtOH) gave an anal. sample with the same mp. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N, S.

**4'-Methylthio-4-nitrostilbene (22).**—A soln of 2 g of 21 in DMSO (10 ml) was refluxed for 3 hr, cooled to room temp, and dild (H<sub>2</sub>O) to give, after recrystn (PhMe), 1.6 g (86%) of the product, mp 172–174°. Mmp with the first product in the previous reaction gave no depression. Recrystn (PhMe) gave an anal. sample, mp 173–174°. *Anal.* (C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub>S) C, H, N, S. This product can also be obtained in high yield from the crude mixed product of the foregoing procedure.

**4'-Methylthio-4-stilbenamine (23).**—Hydrazine hydrate (100%) and Pd/C (5%) reduction of 22 gave the amine (97%), mp 168–169° (EtOH). *Anal.* (C<sub>15</sub>H<sub>13</sub>NS) C, H, N, S.

**N-4-(4'-Methylthiostilbenyl)acetamide (24).**—Acetylation of 23 gave the amide, mp 242.5–243.5°. Recrystn from EtOH–Me<sub>2</sub>CO (1:1) gave mp 243–244°. *Anal.* (C<sub>17</sub>H<sub>17</sub>NOS) C, H, N, S.

## Antitumor Activities and Rates of Hydrolysis of Schiff Bases

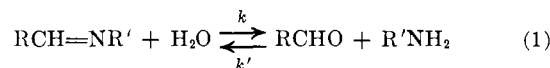
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Although some Schiff bases have shown activity against a variety of animal tumors,<sup>1</sup> many of these compounds hydrolyze rapidly in neutral aq solution near room temp.<sup>2</sup> We have prepared 11 aromatic Schiff bases with electron-withdrawing substituents in order to increase their resistance to hydrolysis and thereby improve their antitumor activities. New compounds are listed in Table I with some of their properties.

The hydrolysis rates of these Schiff bases were determined in H<sub>2</sub>O buffered at pH 7.0 since cellular and intercellular fluids generally have a pH close to 7. At this pH the rate-controlling step in the hydrolysis of most of these compounds is the addition of a molecule of water to the Schiff base<sup>3</sup> and the kinetics of the reaction becomes pseudo first order in the Schiff base. Assuming that



the integrated and simplified rate expression is<sup>4</sup>

$$\frac{x_e}{k(2a - x_e)} \ln \frac{ax_e + x(a - x_e)}{a(x_e - x)} = t \quad (2)$$

where  $a$  = initial concn of Schiff base,  $a - x$  = concn of Schiff base at time  $t$ , and  $a - x_e$  = concn of Schiff base at equilibrium. The plot of  $\log [ax_e + x(a - x_e)]/[a(x_e - x)]$  vs. time should be a straight line for which the slope is  $k(2a - x_e)/2.3x_e$ . The rate constants were calcd by use of eq 2 and the best one was selected by the method of least squares. Since salicylaldehyde, one of the products of the reaction, oxidizes in H<sub>2</sub>O solution under these conditions its absorption in the uv would change gradually. The absorbance of salicylaldehyde at equilibrium was calcd by the method of Guggenheim<sup>5</sup> assuming a first-order oxidation of the salicylaldehyde. Since only the first part of each reaction was used to calculate the rate the slow oxidation of salicylaldehyde did not affect these results. The reaction rate constants for these reactions, given in Table II, indicate

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