## Benzothiazolines as Antituberculous Agents

P. J. PALMER,* R. B. TRIGG, AND J. V. WARRINGTON

*Twyford Laboratories Ltd., London, England*

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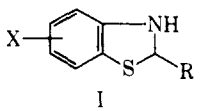
2-Arylbenzothiazoles have been shown by previous workers[1] to have antimicrobial properties, and in particular to be active both *in vitro* and *in vivo* against *Mycobacterium tuberculosis*. It was therefore of interest to prepare a series of benzothiazolines containing aryl or heterocyclic groups in the 2 position, and to examine their biological properties.

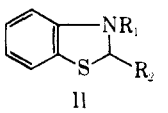
Three types of benzothiazoline (I–III) were synthesized by condensation of an aldehyde or ketone with the appropriately substituted *o*-aminobenzenethiol or its hydrochloride in EtOH. Reaction normally occurred readily either at room temp or on brief warming.

Compounds of type I are readily oxidized to the corresponding benzothiazole, and the physical data quoted in the literature for a number of benzothiazolines would suggest contamination with the oxidation product. In general, we have found that benzothiazolines in which the 2 substituent is electron attracting are considerably more stable than those with electron-donating groups. The latter are often converted into the corresponding benzothiazole simply by crystallization or by treatment with mineral acid.

The assignment of the benzothiazoline structure I to the products obtained was supported[2] by NH stretching bands at 3100–3390 $cm^{-1}$ in their ir spectra and a band ($\epsilon$ 3000–10,000) at 315 ± 5 m$\mu$ in the uv. In contrast, the corresponding benzothiazoles showed strong bands ($\epsilon$ 20,000–25,000) in the region 300–350 m$\mu$. Purity of products was further confirmed by tlc on silica (fluorescent); under uv light, benzothiazoles fluoresced bright blue, whereas the benzothiazolines gave quenched spots. In several cases, benzothiazolines were oxidized to the corresponding benzothiazole using commercial $MnO_2$.




I

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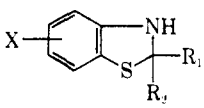


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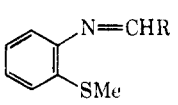



IV

The pyridylbenzothiazolines (I; R = *x*-pyridyl) suffered ring opening[3] when attempts were made to N-methylate them with MeI in methanolic NaOH solution; the product was the azomethine (IV; R = *x*-pyridyl). Acylation of (I; R = 2-pyridyl) with $Ac_2O$ and with BzCl in pyridine gave (II; $R_1$ = Ac, $R_2$ = 2-pyridyl) and (II; $R_1$ = Bz, $R_2$ = 2-pyridyl). These could not be satisfactorily reduced to N-alkylated benzothiazolines with LAH, though a similar compound (II; $R_1$ = Ac, $R_2$ = *p*-diethylaminoethoxyphenyl) gave the corresponding *N*-Et compound in reasonable yield by this procedure. The required N-methylated compounds (II; $R_1$ = Me, $R_2$ = *x*-pyridyl) were prepared by condensation of the appropriate pyridine aldehyde with *o*-methylaminothiophenol. This compound was prepared easily and in high yield by LAH reduction of bis-(2-formamidophenyl) disulfide, rather than by the more tedious literature[4] procedure.

For the synthesis of benzene ring substituted benzothiazolines, pure samples of the appropriately substituted *o*-aminothiophenols were required. These were best obtained, in some cases, by LAH reduction of the corresponding di- or trisulphide, followed by distillation of the free thiol under reduced pressure in $N_2$ atmosphere.

**Biological Activity.**—The benzothiazolines were tested *in vitro* against a range of organisms including Gram-positive and Gram-negative bacteria, dermatophytes, and *Entamoeba histolytica*. A number of compounds showed activity against Gram-positive bacteria and against dermatophytes, but this was not of a sufficiently high order to warrant further study. *In vitro* activity against *M. tuberculosis* (H37Rv) was found for most of the benzothiazolines (see Table I), and because of their ease of preparation and relative stability, the pyridyl compounds (**16–18**) were taken as a basis for further structure–activity study. N-Substitution decreased activity (**23–27**) but introduction of a 2-Ph group (**30**) increased activity as, also, in general, did benzene ring substitution (**34, 36, 37, 39–43, 45, 47–49**). Greatest activity was found for the 6-methoxy compound (**48**), and this and several others were tested for *in vivo* activity.[5] Only slight activity was found for any of the compounds.

### Experimental Section[6]

**General Methods for Preparing Benzothiazolines. Method A.**—The aldehyde, dissolved in an equal vol of MeOH or EtOH was added to an equimolar quantity of *o*-aminobenzenethiol (*o*-ABT) in the same solvent (5 ml/g). The reaction mixt was maintained under the conditions specified when the product had either crystd or was pptd by the careful addn of $H_2O$ (see Table I).

**Method B.**—Equimolar quantities of the N-substituted *o*-ABT and the appropriate aldehyde were heated together for 5 min at 150°, the reaction mixt was cooled and triturated with hexane, and the solid was collected and crystd (see Table II).

**Method C.**—*o*-ABT·HCl (0.04 mole) in EtOH (20 ml) was treated with the appropriate pyridyl ketone (0.04 mole) in EtOH (10 ml), warming briefly if necessary to effect soln. The reaction mixt was maintained under the conditions specified and evapd to dryness under reduced pressure, the residue was dissolvedin $H_2O$, the soln was basified with dil $NH_4OH$, and the product was isolated by $Et_2O$ extn (see Table II).

**Method D.**—The appropriate pyridine carboxaldehyde (0.1 mole) was added to the substituted *o*-ABT·HCl (0.01 mole) in EtOH (10 ml) and the resulting crystals were collected. In some cases, $Et_2O$ (*ca.* 15 ml) was added and the mixt was stored at 0° for 1 hr before collecting the product (see Table III).

**2-(5-Nitro-2-furyl)benzothiazoline.**—5-Nitrofurfural (2.2 g) in $Et_2O$ (100 ml) was added dropwise to a stirred soln of *o*-ABT

(1) (a) W. Logemann, S. Galimberti, G. Tosolini, I. de Carneri, and G. Coppi, *Farmaco Ed. Sci.*, **16**, 795 (1961). (b) B. Prescott and J. M. Webb, *Antibiot. Chemother.*, **8**, 33 (1958). (c) H. D. Cossey, R. N. Gartside, and F. F. Stephens, *Arzneim.-Forsch.*, **16**, 33 (1966).

(2) F. J. Goetz, *J. Heterocycl. Chem.*, **5**, 509 (1968).

(3) *Cf.* F. J. Goetz, *ibid.*, **4**, 80 (1967).

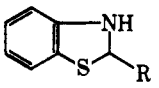
(4) A. I. Kiprianov and Z. N. Pazenko, *Zh. Obshch. Khim.*, **19**, 1523 (1949); *Chem. Abstr.*, **44**, 3488 (1950).

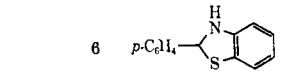
(5) *In vivo* screening was done using a slight modification to the method of G. P. Youmans and A. S. Youmans, *Amer. Rev. Tuberc.*, **64**, 541 (1951).

(6) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points were determined with a Büchi apparatus using open capillary tubes. Ir spectra were recorded for Nujol mulls on a Perkin-Elmer 237 spectrophotometer. Uv spectra were determined for EtOH solutions.

TABLE I

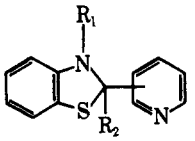
PREPARED BY METHOD A



| No. | R | Solvent | Time, min | Temp, °C | Yield, % | Mp, °C | Cryst solvent | Formula | Analysis | MIC[l] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ph | EtOH | 30 | 25 | 52 | 76–77[a] | $Et_2O$–hexane | $C_{13}H_{11}NS$ | C, H, N | 200 |
| 2 | *m*-Cyanophenyl | EtOH | 60 | 25 | 40 | 92–94 | EtOH–$H_2O$ | $C_{14}H_{10}N_2S$ | C, H, N, S | 6.25 |
| 3 | *p*-Nitrophenyl | MeOH | 30 | 25 | 63 | 119.5–121 | MeOH | $C_{13}H_{10}N_2O_2S$ | C, H, N, S | >200 |
| 4 | Piperonyl | MeOH | 960 | 0[b] | 43 | 55–56 | [c] | $C_{14}H_{11}NO_2S$ | [c] | 200 |
| 5 | *p*-$C_6H_4OCH_2CH_2NEt_2$ | EtOH | 30 | 25 | 71 | 63–64 | $C_6H_6$–hexane | $C_{19}H_{29}N_2OS$ | C, H, N | 12.5 |
| 6 |  | EtOH | 10 | 25 | 90 | 138–140 | $Me_2CO$–hexane | $C_{16}H_{16}N_2S_2$ | C, H, N, S | >200 |
| 7 | Benzyl | EtOH | 30 | 78 | 48[d] | 61–62 | $C_6H_6$–hexane | $C_{14}H_{13}NS$ | C, H, N, S | 50 |
| 8 | Ferrocenyl | MeOH | 15 | 25 | 79 | 128–130 | EtOH | $C_{17}H_{15}FeNS$ | C, H, N, S | 0.8 |
| 9 | Undecenyl | MeOH | 2 | 65 | 51 | 46.5–47.5 | MeOH | $C_{17}H_{25}NS$ | C, H, N | 100 |
| 10 | 2-Benzothiazolyl | EtOH | 960 | 25 | 84[e] | 115.5[f] | $C_6H_6$–hexane | $C_{14}H_{10}N_2S_2$ | C, H, N, S | 50 |
| 11 | 2-Benzimidazolyl | See Experimental Section | | | 53 | 169.5–172[g] | EtOH–$H_2O$ | $C_{14}H_{11}N_3S \cdot 0.5H_2O$[g] | C, H, N, S | 25 |
| 12 | 2-Furyl | EtOH | 30 | 25 | 99 | Oil[h] | | $C_{11}H_9NOS$ | [h] | 3.1 |
| 13 | 5-Nitro-2-furyl | See Experimental Section | | | 75 | 121–122 | $C_6H_6$–hexane | $C_{11}H_8N_2O_3S$ | C, H, N | 6.3 |
| 14 | 2-Pyrrolyl | EtOH | 960 | 25 | 55 | 83.5–85 | $C_6H_6$–hexane | $C_{11}H_9N_2S$ | C, H, N, S | 0.6 |
| 15 | 2-Quinolyl | MeOH | 30 | 25 | 96 | 107.5–109 | $C_6H_6$–hexane | $C_{18}H_{12}N_2S$ | C, H, S; N[k] | 1.6 |
| 16 | 2-Pyridyl | EtOH | 5 | 60 | 73 | 99–101 | $Me_2CO$ | $C_{12}H_{10}N_2S$ | C, H, N, S | 2.2 |
| 17 | 3-Pyridyl | EtOH | 5 | 60 | 73 | 89–91 | $Me_2CO$ | $C_{12}H_{10}N_2S$ | C, H, N, S | 4.4 |
| 18 | 4-Pyridyl | EtOH | 5 | 60 | 57 | 103–104 | $Me_2CO$ | $C_{12}H_{10}N_2S$ | C, H, N, S | 6.25 |
| 19 | 2-Pyridyl *N*-oxide | MeOH | 15 | 25 | 43 | 179–180 | EtOH–hexane | $C_{12}H_{10}N_2OS$ | C, H, N, S | 6.25 |
| 20 | 4-Hydroxy-3-pyridyl | EtOH | 2 | 25 | 92 | 226.5–228[i] | [j] | $C_{12}H_{11}ClN_2OS$ | C, H, N, S | 1.6 |
| 21 | 6-Methyl-2-pyridyl | See Experimental Section | | | 65 | 93.5–94.5 | $C_6H_6$–hexane | $C_{13}H_{12}N_2S$ | C, H, N, S | 3.1 |
| 22 | *N*-Methyl-2-pyridinium iodide | EtOH | 2 | 78 | 85[k] | 134.5–136 | EtOH–$Et_2O$ | $C_{13}H_{13}IN_2S$ | N | |

[a] Lit. [M. Claasz, *Chem. Ber.*, **49**, 1141 (1916)] gives mp 108–109°. [b] Reaction mixt allowed to warm to 25° during reaction period. [c] Compd unstable; further characterized by oxidn to known [M. T. Bogert and A. Stull, *J. Amer. Chem. Soc.*, **47**, 3078 (1925)] 2-piperonylbenzothiazole. [d] After chromatog on $SiO_2$ using hexane–$C_6H_6$ (1:9). [e] After chromatog on $SiO_2$ using $C_6H_6$. [f] Resolidifies and remelts at 280–300°; has a lower melting form, mp 93–94°, identical spectroscopically with higher mp form. [g] Resolidifies and remelts at 270–280°; $H_2O$ of cryst confirmed by ir spec. [h] Bp 128–138° (0.01 mm); compd unstable; further characterized by oxidn to known[c] 2-furylbenzothiazole. [i] Free base unstable; prepd using *o*-aminothiophenol·HCl; isolated as HCl salt which was washed with hot EtOH. [j] Pptd with $Et_2O$; product deteriorates rapidly on storage; [k] N: calcd 10.6; found 10.0. [l] MIC values quoted in Tables I–III are in μg/ml and were detd by serial diln in Proskauer and Beck medium. Isoniazid had MIC 0.04–0.08.

TABLE II



| No. | $R_1$ | $R_2$ | *x*-Pyridyl | Method | Time, hr | Temp, °C | Yield, % | Mp, °C | Cryst solvent | Formula | Analysis | Salts | MIC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | Me | H | 2 | B | | | 62 | 61–62 | EtOH–$H_2O$ | $C_{13}H_{12}N_2S$ | H, N; C[a] | *a* | 12.5 |
| 24 | Me | H | 3 | B | | | 65 | 98–99 | EtOH–$H_2O$ | $C_{13}H_{12}N_2S$ | C, H, N | *b* | 50 |
| 25 | Me | H | 4 | B | | | 56 | 121.5–123 | EtOH–$H_2O$ | $C_{13}H_{12}N_2S$ | C, H, N | | 25 |
| 26 | Et | H | 2 | B | | | Quant | Oil | | $C_{14}H_{14}N_2S$ | *c* | *c* | |
| 27 | Et | H | 4 | B | | | 72 | 62.5–65[d] | EtOH–$H_2O$ | $C_{14}H_{14}N_2S$ | C, H, N | *d* | 25 |
| 28 | H | Me | 2 | C | 20 | 0 | 73 | 96.5–98 | $Me_2CO$ | $C_{13}H_{12}N_2S$ | C, H; N[e] | | 6.3 |
| 29 | H | Et | 2 | C | 0.5 | 25 | 87 | 76–78 | $Me_2CO$ | $C_{14}H_{14}N_2S$ | C, H, N | | >200 |
| 30 | H | Ph | 2 | C | 20 | 0 | 71 | 118–119 | $Me_2CO$ | $C_{18}H_{14}N_2S$ | C, H, N | | 0.6 |
| 31 | H | 2-Pyridyl | 2 | C | 0.5 | 25 | 87 | 139–140 | MeOH | $C_{17}H_{13}N_3S$ | C, H, N, S | | 12.5 |
| 32 | H[f] | Me | 2 | C | 0.2 | 25 | 44 | 196–199 | MeOH–$Et_2O$ | $C_{14}H_{15}ClN_2S$ | C, H, N, Cl | | 1.6 |

[a] C: calcd, 68.4; found 68.85; HCl salt, mp 179–182° dec (EtOH–$Et_2O$) $C_{13}H_{13}ClN_2S$ (C, H, N). [b] HCl salt, mp 174.5–176.5° (EtOH). $C_{13}H_{13}ClN_2S$ (C, H, N). [c] Characterized as picrate, mp 149–150° dec (EtOH), $C_{20}H_{17}N_5O_7S$ (C, H, N). [d] Bp 146–152° (0.015 mm); picrate, mp 146–8° (EtOH), $C_{20}H_{17}N_5O_7S$ (C, H, N). [e] N: calcd, 12.3; found, 11.7. [f] 5-Me group in benzene ring; isolated and characterized as HCl salt.

(2.0 g) in $Et_2O$ (100 ml) at −60° under $N_2$. The soln was allowed to warm to 25° overnight, the bulk of the solvent removed *in vacuo* at 25° and the residue triturated with petr ether (see Table I).

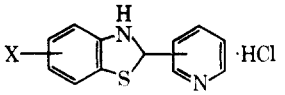
**2-(6-Methyl-2-pyridyl)benzothiazoline and -benzothiazole.**—A soln of 6-methylpyridine-2-carboxaldehyde (0.97 g) in EtOH (1 ml) was added to *o*-ABT (1.0 g) in EtOH (5 ml). The resulting soln was allowed to stand for 1 hr at 25°, refluxed for 2 min, and cooled to 0°, to give pale yellow crystals (30%) of 2-(6-methyl-2-pyridyl)benzothiazole: mp 148–149° ($C_6H_6$–hexane): $\lambda_{max}$ 313 mμ (ε 22,100). *Anal.* ($C_{13}H_{10}N_2S$) C, H, N. On further standing, the filtrate deposited the required benzothiazoline (65%) (see Table I). Condensation of *o*-ABT·HCl with 6-methylpyridine-2-carboxaldehyde in EtOH for 1 hr at 25° gave, by pptn with $Et_2O$, **2-(6-methyl-2-pyridyl)benzothiazoline·HCl**, mp 184–185° (EtOH–$Et_2O$). *Anal.* ($C_{13}H_{13}ClN_2S$) C, H, N, S.

**2-(2-Benzimidazolyl)benzothiazoline.**—Benzimidazole-2-carboxaldehyde (0.5 g) was dissolved in the min vol of 2 *N* HCl and the soln was dild with EtOH (10 ml) and treated with a soln of *o*-ABT·HCl (0.55 g) in EtOH (5 ml). The soln was refluxed for 2 min, cooled, basified with $NH_4OH$, and dild with $H_2O$. The resulting solid was crystd to give the required compd (see Table I).

**3-Acetyl-2-(2-pyridyl)benzothiazoline.**—$Ac_2O$ (8 ml) was added to a soln of **16** (4.1 g) in pyridine (8 ml) and the soln was kept at 25° for 18 hr. Working up in the usual way gave an oil (4.5 g). A sample for analysis was distd at 0.015 mm. *Anal.* ($C_{14}$-

TABLE III

PREPARED BY METHOD D



| No. | X | x-Pyridyl | Yield, % | Mp, °C | Cryst solvent | Formula | Analysis | MIC |
|---|---|---|---|---|---|---|---|---|
| 33 | 5-Me | 2 | 89 | 197 | EtOH–$Et_2O$ | $C_{13}H_{13}ClN_2S$ | C, H, N, Cl | 3.1 |
| 34 | 5-Me | 3 | 81 | 161–170 | EtOH–$Et_2O$ | $C_{13}H_{13}ClN_2S$ | C, H, N, Cl | 3.1 |
| 35 | 5-Me | 4 | 42 | 261–268 | EtOH–$Et_2O$ | $C_{13}H_{13}ClN_2S$ | C, H, N, Cl | 50 |
| 36 | 5-Cl | 2 | 92 | 202–203 | EtOH–$Et_2O$ | $C_{12}H_{10}Cl_2N_2S$ | C, H, N; Cl[c] | 0.8 |
| 37 | 5-Cl | 3 | 79 | 196–197 | MeOH–$Et_2O$ | $C_{12}H_{10}Cl_2N_2S$ | C, H, N, Cl | 1.6 |
| 38 | 5-Cl | 4 | 94 | 225–254 | MeOH–$Et_2O$ | $C_{12}H_{10}Cl_2N_2S$ | C, H, N, Cl | 12.5 |
| 39 | 5-$CF_3$ | 2 | 82 | 163–164 | MeOH–$Et_2O$ | $C_{13}H_{10}ClF_3N_2S$ | H, N, Cl; C[d] | 1.6 |
| 40 | 5-$CF_3$ | 3 | 88 | 148–157 | MeOH–$Et_2O$ | $C_{13}H_{10}ClF_3N_2S$ | C, H, N, Cl | 3.1 |
| 41 | 5-$CF_3$ | 4 | 14 | 137–141 | EtOH–$Et_2O$ | $C_{13}H_{10}ClF_3N_2S$ | H, N, Cl; C[e] | 1.0 |
| 42 | 5-$NO_2$ | 2 | 85 | 126.5–128[a] | EtOH–$Et_2O$ | $C_{12}H_9N_3O_2S$ | C, H, N | 1.25 |
| 43 | 5-$NO_2$ | 3 | 91 | 187–188[a] | EtOH | $C_{12}H_9N_3O_2S$ | C, H, N | 0.4 |
| 44 | 6-Cl | 2 | 77 | 186–187 | EtOH–$Et_2O$ | $C_{12}H_{10}Cl_2N_2S$ | C, H, N, Cl | 3.1 |
| 45 | 6-Cl | 3 | 90 | 192–193 | EtOH–$Et_2O$ | $C_{12}H_{10}Cl_2N_2S$ | C, H, N, Cl | 1.0 |
| 46 | 6-Cl | 4 | 88 | 128.5–131[a] | MeOH | $C_{12}H_9ClN_2S$ | C, H, N, Cl | 6.3 |
| 47 | 6-MeO | 2 | 85 | 194–195 | EtOH–$Et_2O$ | $C_{13}H_{13}ClN_2OS$ | C, H, N, Cl | 1.9 |
| 48 | 6-MeO | 3 | 96 | 194–196 | EtOH–$Et_2O$ | $C_{13}H_{13}ClN_2OS$ | C, H, N, Cl | 0.16 |
| 49 | 6-MeO | 4 | 92 | 183[b] | EtOH–$Et_2O$ | $C_{13}H_{13}ClN_2OS$ | C, H, Cl | 1.6 |

[a] Free base. [b] Resolidifies and remelts 232°. [c] Cl: calcd, 24.9; found, 23.8. [d] C: calcd, 49.0; found, 48.4. [e] C: calcd, 49.0; found, 50.0.

$H_{12}N_2OS$) C, H, N. The compd gave a hydrochloride, mp 177–183° (MeOH–$Et_2O$). *Anal.* ($C_{14}H_{13}ClN_2OS$) C, H, N.

**3-Benzoyl-2-(2-pyridyl)benzothiazoline.**—To a soln of **16** (2.14 g) in $Et_2O$ (50 ml) was added a soln of BzCl (1.41 g) in $Et_2O$ (15 ml). The mixt was stirred at 25° for 1.5 hr and filtered and the filtrate treated with $Et_2O$–HCl to give a solid (1.47 g), mp 125–137° dec. Conversion into the free base gave the compd: mp 110–112° (MeOH): $\nu_{max}$ 1672 $cm^{-1}$. *Anal.* ($C_{19}H_{14}N_2OS$) H, N; C: calcd, 71.7; found 71.1.

**Attempted N-Methylation of 2-(Pyridyl)benzothiazolines.**—A soln of **17** (1.5 g) in MeOH (15 ml) was treated with 40% NaOH soln (0.7 ml; 1 equiv). To the red soln was added MeI (0.44 ml; 1 equiv) and after 30 min the reaction mixt was poured into $H_2O$ and the product was isolated by $Et_2O$ extn to give IV (R = 3-pyridyl): mp 67–68.5° (hexane); $\lambda_{max}$ 206.5, 253, 268 (sh), and 367.5 (br) ($\epsilon$ 25,400, 21,100, 18,700, and 4200, resp). *Anal.* ($C_{13}H_{12}N_2S$) C, H, N. The same compd was obtained when equimolar amts of *o*-methylthioaniline and pyridine-3-carboxaldehyde were heated for 30 min in EtOH.

Similar reaction of MeI with **16** and **18** gave IV (R = 2-pyridyl), mp 87–88° (hexane). *Anal.* ($C_{13}H_{12}N_2S$) C, H, N and (IV; R = 4-pyridyl) mp 84.5–85.5° (hexane). *Anal.* ($C_{13}H_{12}N_2S$) C, H, N.

**3-Ethyl-2-*p*-(β-diethylaminoethoxy)phenylbenzothiazoline.**—Compd **5** (3.28 g) in $Et_2O$ (15 ml) contg $Ac_2O$ (1.41 ml) was left overnight at 25°. The oily product with $Et_2O$–HCl gave a solid (1.8 g), mp 162–164° (EtOH–$Et_2O$). Conversion into the free base and redn with LAH (1 g) in $Et_2O$ (100 ml) gave the compd as an oil (1.06 g) which gave a citrate, mp 100–102° (EtOH–$Et_2O$). *Anal.* ($C_{27}H_{36}N_2O_8S$) H, N; C: calcd, 59.1; found, 58.6.

**2-(2-Pyridyl)-5-methylbenzothiazole.**—Pyridine-2-carboxaldehyde (1.07 g) and 2-amino-4-methylbenzenethiol (1.39 g) were refluxed in $PhNO_2$ (10 ml) for 15 min, the solvent was removed by steam distn, the residue was extd into $C_6H_6$, and the $C_6H_6$ soln was filtered through a column of alumina. Elution with $C_6H_6$ gave a solid (0.66 g), mp 150–154°, raised by crystn (EtOH) to 152–155°. *Anal.* ($C_{13}H_{10}N_2S$) C, H, N.

**2-(2-Pyridyl)-5-chlorobenzothiazole.**—Compd **36** (1.0 g) was converted into the free base, and to a soln of this in $Me_2CO$ (20 ml) was added a slight excess of Jones' chromic acid soln.[7] Concn to small bulk and isolation of the product in the usual way gave a solid (400 mg) which, after several crystns from EtOH, gave the benzothiazole, mp 170–172°. *Anal.* ($C_{12}H_7ClN_2S$) C, H, N.

**2-(3-Indolyl)benzothiazole.**—*o*-ABT (1.64 g) and indole-3-carboxaldehyde (2.0 g) in EtOH (20 ml) contg 2 drops of concd HCl was refluxed 30 min, cooled, neutralized with $NH_4OH$, and dild with $H_2O$ to give a solid (1.87 g): mp 169.5–170.5° ($C_6H_6$–hexane); $\lambda_{max}$ 333 mμ ($\epsilon$ 27,600). *Anal.* ($C_{15}H_{10}N_2S$) C, H, N.

**2-Amino-4-methylbenzenethiol.**—3-Nitro-4-chlorotoluene (51.5 g) was added during 20 min to a stirred soln of sodium polysulfide prepd from S (57.6 g), $Na_2S \cdot 9H_2O$ (216 g), and $H_2O$ (200 ml) at 70°. The reaction mixt was stirred at 90–95° for 2 hr, EtOH (100 ml) added, and heating continued for a further 1 hr before removing the solvent by steam distn. The residue was cooled to 5° and the solid (A; 11.5 g), mp 132–134°, collected. The filtrate was acidified (50% AcOH) and $Et_2O$ extd to give a residue (30 g) of S and an $Et_2O$-sol solid (B; 19.0 g), mp 96–130°. Crystn of A from MeOH gave bis(2-amino-4-methylphenyl) trisulfide, mp 135–136°. *Anal.* ($C_{14}H_{16}N_2S_3$) C, H, S; N: calcd, 9.1; found 9.8.

A soln of either A or B (5.52 g) in a mixt of THF (40 ml) and $Et_2O$ (30 ml) was added to LAH (2.5 g) in $Et_2O$ (50 ml), the mixt was refluxed for 1 hr, cooled, and decompd with $H_2O$ and dil HCl. The $Et_2O$ layer was dried and treated with HCl–$Et_2O$ to give the title compound as its hydrochloride (5.9 g), mp 187–189°. The free base had mp 42–44°, bp 92–93° (2 mm). *Anal.* ($C_7H_9NS$) C, H, N. Oxidn of the amine in 50% HCl with 50% $FeCl_3$ soln gave bis(2-amino-4-methylphenyl) disulfide, mp 72–73° (EtOH). *Anal.* ($C_{14}H_{16}N_2S_2$) C, H, N, S.

**2-Amino-4-chlorobenzenethiol.**—Reaction of 2,5-dichloronitrobenzene (57.5 g) with sodium polysulfide as above gave, after adjusting the pH of the reaction mixt to 7 with AcOH, a solid (75.9 g). This was dissolved in hot EtOH (500 ml), the soln filtered, and the filtrate concd to give successive crops of solid: A, mp 129–134° (9.95 g); B, mp 110–139° (4.4 g); C, mp 134–138° (2.53 g); and a final crop D, (31 g of gummy solid). Crystn of C (EtOH–MeCO) gave bis(2-amino-4-chlorophenyl) trisulfide, mp 142.5–143.5°. *Anal.* ($C_{12}H_{10}Cl_2N_2S_3$) C, H, N, S.

Redn of D (5 g) with LAH (2 g) in $Et_2O$ (200 ml) using the procedure described above gave the required compd, mp 44–46°, bp 102° (1.8 mm). *Anal.* ($C_6H_6ClNS$) C, H, N, Cl, S.

**2-Amino-5-chlorobenzenethiol.**—6-Chloro-1,3,2-benzothiazathionium chloride[8] (140 g) was added portionwise to $H_2O$ (2 l.) and after 2 hr, $Et_2O$ (1 l.) was added and the mixt was stirred for 0.5 hr longer. The $Et_2O$ layer was sepd, the $H_2O$ layer was extd with $Et_2O$ (2 × 1 l.), and the $Et_2O$ exts were combined, washed, and dried to afford a purple solid (99 g), mp 112–114° dec. This solid in MeOH (1 l.) was added to a stirred soln of NaOH (100 g) in $H_2O$ (1 l.) at 10–15°, the mixt allowed to warm to 25°, the pH adjusted to 7 with 50% AcOH, and the product extd into $Et_2O$. Addn of $Et_2O$–HCl gave the thiol·HCl (67.5 g), mp 210–212°. The free base had mp 76–79° ($C_6H_6$–hexane), bp 85–86° (0.2 mm).

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*Anal.* ($C_6H_6ClNS$) C, H, Cl, N, S. The thiol readily oxidized in air to give bis(2-amino-5-chlorophenyl) disulfide, mp 109–110° (MeOH). *Anal.* ($C_{12}H_{10}Cl_2N_2S_2$) C, H, N, Cl; S: calcd 20.2; found, 20.8%.

**2-Amino-5-methylbenzenethiol.**—2-Amino-6-methylbenzothiazole (52 g), KOH (160 g), and $H_2O$ (320 ml) were refluxed 8 hr under $N_2$. The soln was cooled and the pH adjusted to 7 with 50% AcOH to afford a solid (44 g). Cleavage of this with LAH as before gave the thiol, mp 68–70°, bp 91° (2 mm). *Anal.* ($C_8H_7NS$) C, H, N, S. Oxidation with $FeCl_3$ gave the corresponding disulfide, mp 88° (EtOH). *Anal.* ($C_{14}H_{16}N_2S_2$) C, H, N, S.

**2-Amino-4-trifluoromethylbenzenethiol.**—Redn of bis(2-amino-4-trifluoromethylphenyl) disulfide[9] (4.5 g) with LAH as above gave the compd, bp 77–78° (2 mm), $n^{25}D$ 1.5332. Despite several attempts, the compd failed to analyze satisfactorily, possibly due to its high F content. *Anal.* Calcd for $C_7H_6F_3NS$; C, 43.5; H, 3.1; N, 7.25; S, 16.6; F, 29.5. Found: C, 44.7; H, 3.8; N, 7.1; S, 15.85; F, 29.0.

***o*-Methylaminobenzenethiol.**—Bis(2-formamidophenyl) disulfide[10] (5.0 g) in $Et_2O$ (150 ml) was added to LAH (5.0 g) in $Et_2O$ (50 ml) and the soln was refluxed 1 hr, cooled, and decompd under $N_2$ with 2 *N* HCl. The $Et_2O$ layer was washed, dried, and distd to give the thiol (4.18 g), bp 127–128° (18 mm).

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## Linear Polypeptides of a Known Sequence of Amino Acids. Synthesis and Immunochemical Properties of Poly(L-tyrosyl-L-glutamylglycylglycyl)glycine Methyl Ester

BRIAN J. JOHNSON* AND ELWOOD G. TRASK

*Department of Chemistry, Tufts University, Medford, Massachusetts 02155*

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A recent investigation of the immunochemical properties of poly(Try-Glu-Gly-Gly)Gly-*1*-$^{14}C$ Et ester[1,2] has demonstrated that the polypeptide is antigenic, eliciting antibodies in rabbits.[3]

It has been shown that the alanyl residue is not part of the active site of this antigen.[4] However, it was unknown if the alanyl residue played any role in the antigenicity of the polypeptide. To investigate this latter point it was considered that substitution of the alanyl residue with the sterically smaller and optically inactive glycyl residue may affect the immunochemical properties of the molecule. To this end we wish to report the synthesis and immunochemical properties of poly(Tyr-Glu-Gly-Gly)glycine Me ester (**1**).

**Chemistry.**—Z-γ-*tert*-butylglutamic acid pentachlorophenyl ester was coupled to Gly-Gly Me·HCl[5] to yield Z-γ-*tert*-Bu-Glu-Gly-Gly Me ester. Catalytic hydrogenolysis of the fully protected tripeptide in the presence of dry HCl produced γ-*tert*-Bu-Glu-Gly-Gly Me·HCl (**2**). This material was coupled to Z-*O*-*tert*-Bu-tyrosine pentachlorophenyl ester[1] to give the fully protected tetrapeptide, Z-*O*-*tert*-Bu-Tyr-γ-*tert*-Bu-Glu-Gly-Gly Me ester (**3**). Hydrolysis of **3** with 1 *N* NaOH in MeOH gave the free acid, Z-*O*-*tert*-Bu-Tyr-γ-*tert*-Bu-Glu-Gly-Gly (**4**), which was coupled to pentachlorophenol using 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl *p*-toluenesulfonate to obtain the tetrapeptide active ester, Z-*O*-*tert*-Bu-Tyr-γ-*tert*-Bu-Glu-Gly-Gly pentachlorophenyl ester (**5**). The carbobenzoxy protecting group was removed by catalytic hydrogenolysis in the presence of dry HCl to give the polymerizing unit *O*-*tert*-Bu-Tyr-γ-*tert*-Bu-Glu-Gly-Gly pentachlorophenyl ester·HCl (**6**). The polymerization was performed at a reagent concentration of 100 mmoles/l. in the presence of a performed monomer, since this has been shown to produce linear high mol wt polypeptides.[1,2,6-8] Following this established procedure, the insoluble polymer, poly(*O*-*tert*-Bu-Tyr-γ-*tert*-Bu-Glu-Gly-Gly)Gly methyl ester was prepared, from which the protecting *tert*-Bu groups were removed by the use of 90% $F_3CCO_2H$ to yield poly(Tyr-Glu-Gly-Gly)Gly methyl ester (**1**). After extensive dialysis, the polymer was purified and fractionated by filtration through calibrated columns of Sephadex G-100[9] and Corning CPG 10-240 glass granules. By this means the mol wt of the polypeptide was found to be at least $1 \times 10^5$.

**Immunochemistry.**—Two rabbits were immunized with poly(Tyr-Glu-Gly-Gly)Gly methyl ester (**1**) using the same protocol as that previously described.[3] To aliquots of the sera obtained from each rabbit was added up to 10,000 μg of the synthetic polypeptide **1**. No precipitin reaction was observed.

The possibility that the polypeptide **1** could be a hapten for the antigen poly(Tyr-Glu-Ala-Gly)Gly-*1*-$^{14}C$ Et was also investigated. Incremental amounts of the polypeptide poly(Tyr-Glu-Gly-Gly)Gly Me **1** were added to aliquots of antisera to poly(Tyr-Glu-Ala-Gly)Gly-*1*-$^{14}C$ Et containing a known amount of the antigen. No inhibition of the precipitin reaction was observed.

**Conclusions.**—Replacement of the alanyl moiety in poly(Tyr-Glu-Ala-Gly)Gly-*1*-$^{14}C$ Et by the sterically smaller and optically inactive glycyl residue caused a loss in antigenicity. Thus, although the alanyl residue is believed not to be part of the active site of the antigen[4] this residue does confer antigenicity to the molecule. This is most probably achieved by the alanyl residue maintaining such a favorable conformation that the antigenic determinants are made available. The conformation of the polypeptide poly(Tyr-Glu-Gly-Gly)Gly Me is thought to be very different since this polymer does not inhibit the precipitin reaction between the antigen and its antisera.

### Experimental Section

Melting points were taken with a Mel-Temp apparatus and are uncorrected. Optical rotations were taken with a Carl Zeiss precision polarimeter.

**γ-*tert*-Bu-Glu-Gly-Gly Me·HCl (2).**—To a mixture of 10.0 g (0.055 mole) of Gly-Gly Me·HCl in 300 ml of $CH_2Cl_2$ was added 5.55 g (0.055 mole) of $Et_3N$ and 29.25 g (0.05 mole) of Z-γ-*tert*-butylglutamic acid pentachlorophenyl ester. The mixture was stirred overnight at room temp, then coned *in vacuo*. The solid residue was dissolved in EtOAc and washed with 10% citric acid

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