(28) Report by the Methods of Analysis Subcommittee of the Society of Cosmetic Chemists of Great Britain, *J. Soc. Cosmetic Chemists*, **19**, 213(1968).

(29) P. Becher, *J. Phys. Chem.*, **66**, 374(1962).

(30) J. T. Martin and H. A. Standing, *J. Textile Inst.*, **40**, T689 (1949).

(31) L. K. J. Tong and M. C. Glesmann, *J. Am. Chem. Soc.*, **79**, 4310(1957).

(32) *Ibid.*, **78**, 583(1956).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 4, 1969 from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214* and *Westwood Pharmaceuticals, Buffalo, NY 14213*

Accepted for publication August 7, 1969.

* Present address: Dept. of Pharmacy, University of Sydney, Sydney N.S.W. 2006, Australia.

† Present address: Smith Kline & French Laboratories, 1500 Spring Garden Street, Phila., PA 19101

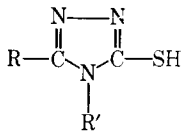
# New 1,2,4(H)-Triazole Derivatives as Diuretic Agents

M. H. SHAH, M. Y. MHASALKAR, V. M. PATKI, C. V. DELIWALA*, and U. K. SHETH

**Abstract** □ Sixty-three new 1,2,4(H)-triazole derivatives have been prepared and their diuretic activity studied in rats. Sequential screening showed 14 compounds possessing significant diuretic activity. 3-Phenyl-4-allyl-5-mercapto-1,2,4(H)-triazole and 3-*o*-chlorophenyl-4-allyl-5-mercapto-1,2,4(H)-triazole were the most potent compounds in the present series.

**Keyphrases** □ Diuretic activity—1,2,4(H)-triazole derivatives □ Mercapto-triazoles—synthesis □ Structure-activity relationship—triazole rings

In a previous communication (1), the authors reported the diuretic activity of some 1,2,4(H)-triazoles (I). Recently, Yale and Piala (2) have also reported the diuretic properties of some *s*-triazole derivatives amongst which 3-(*p*-aminophenyl)-*s*-triazole-5-thiol (I, R = *p*-$NH_2$-$C_6H_4$ and R′ = H) has been claimed to possess good diuretic activity. In view of these interesting results the work has now been extended to some more 3,4-disubstituted-5-mercapto-1,2,4(H)-triazoles.



I

The mercapto-triazoles were synthesized from the corresponding thiosemicarbazides by cyclization with sodium hydroxide or sodium carbonate. Some triazole derivatives were obtained directly in one step from acid hydrazides and the isothiocyanates by heating in excess alkali. When this reaction was carried out at room temperature, it proceeded only as far as the formation of the 1,4-disubstituted thiosemicarbazides.

The list of triazoles prepared, their melting points, yields, analytical data, and diuretic activity are given in Table I.

The requisite thiosemicarbazides were obtained by the reaction of acid hydrazides and isothiocyanates by literature methods. The new thiosemicarbazides are listed in Table II along with their melting points and analytical data.

Since many sulfamoyl compounds are being used clinically as potent diuretics, the conversion of some of the 5-mercapto-1,2,4(H)-triazoles into the corresponding 5-sulfamoyl derivatives was attempted by the usual oxidative chlorination followed by the action of ammonia (3, 4). The 5-sulfamoyl derivatives were obtained in two cases while in some other instances the desired compounds could not be isolated due to extensive decomposition. Moreover, the two sulfamoyl derivatives thus obtained showed activity of lower order than the parent mercapto compounds, *cf.* Yale and Piala (2), hence the preparation of other sulfamoyl derivatives was not pursued.

## PHARMACOLOGY

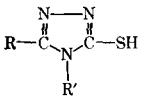
All the 3,4-disubstituted-5-mercapto-1,2,4-triazoles were screened for the diuretic properties in rats at their optimal responsive dose levels by the sequential method of Modi *et al.* (5).

**Method**—Albino rats (male) weighing about 180–200 g. were taken in groups of four in each cage per test dose. Prior to the experiment the rats were allowed food and water *ad libitum*. During the experiment each group of four animals was housed in an improved metabolism cage described by Modi *et al* (6). One group was used as untreated control and received orally the vehicle only, consisting of 0.5 ml. of 2% starch solution. Another group received hydrochlorothiazide (2.5 mg./kg.) as reference compound, suspended in the vehicle. The other groups received the various test compounds in the same vehicle. The urine was collected for 24 hr. If the total volume of urine in Cage I exceeded 19.3 ml., the compound was considered active and if below 3.7 ml., inactive. However, if the volume was in between these two values, a further evaluation with another cage of four rats was made. If the total volume of urine in Cages I plus II exceeded 30.8 ml. the compound was considered active but if less than 15.2 ml. it was considered inactive. In case the volume was again between the two limits a third cage was taken and similarly a fourth one if necessary as per criteria in Table III.

The compounds that did not meet activity criteria in the fourth cage experiment were given up as not sufficiently active.

Among the compounds with acceptable activity, those which produced urinary volumes more than 125% of controls were selected

Table I—3,4-Disubstituted-5-mercapto-1,2,4(H)-triazoles



| Compd. No. | R | R′ | Yield, % | M.p., °C. | Molecular Formula | —Nitrogen, %— Found | Calcd. | Diuretic Activity Opt. Res. Dose | Status |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $C_6H_5$ | $C_2H_5$ | 90 | 141–142 | $C_{10}H_{11}N_3S$ | 20.99 | 20.48 | 3 | Active |
| 2 | $C_6H_5$ | $CH_2CH{=}CH_2$ | 85 | 120–121 | $C_{11}H_{11}N_3S$ | 19.27 | 19.35 | 5 | Active |
| 3 | $C_6H_5$ | $n$-$C_3H_7$ | 85 | 127–128 | $C_{11}H_{13}N_3S$ | 18.82 | 19.18 | 3 | Active |
| 4 | $C_6H_5$ | iso-$C_3H_7$ | 95 | 193–194 | $C_{11}H_{13}N_3S$ | 19.64 | 19.18 | 13 | Active |
| 5 | $C_6H_5$ | $n$-$C_4H_9$ | 50 | 130–131 | $C_{12}H_{15}N_3S$ | 18.13 | 18.02 | 20 | Active |
| 6 | $C_6H_5$ | iso-$C_4H_9$ | 83 | 178–179 | $C_{12}H_{15}N_3S$ | 18.19 | 18.02 | 0.6 | Active |
| 7 | $C_6H_5$ | $C_6H_{11}$ | 84 | 193 | $C_{14}H_{17}N_3S$ | 16.01 | 16.21 | 11 | Active |
| 8 | $C_6H_5$ | $C_6H_5$ | 87 | 277–278 | $C_{14}H_{11}N_3S$ | 16.77 | 16.60 | 5 | Active |
| 9 | 2-$ClC_6H_4$ | $C_2H_5$ | 87 | 194–195 | $C_{10}H_{10}ClN_3S$ | 17.31 | 17.54 | 3 | Inactive |
| 10 | 2-$ClC_6H_4$ | $CH_2CH{=}CH_2$ | 85 | 147–148 | $C_{11}H_{10}ClN_3S$ | 16.50 | 16.70 | 10 | Active |
| 11 | 2-$ClC_6H_4$ | $n$-$C_3H_7$ | 86 | 166–167 | $C_{11}H_{12}ClN_3S$ | 16.35 | 16.57 | 10 | Inactive |
| 12 | 2-$ClC_6H_4$ | iso-$C_3H_7$ | 89 | 206–208 | $C_{11}H_{12}ClN_3S$ | 16.66 | 16.57 | 6 | Inactive |
| 13 | 2-$ClC_6H_4$ | $n$-$C_4H_9$ | 91 | 154–155 | $C_{12}H_{14}ClN_3S$ | 15.49 | 15.70 | 6 | Inactive |
| 14 | 2-$ClC_6H_4$ | iso-$C_4H_9$ | 82 | 157–158 | $C_{12}H_{14}ClN_3S$ | 15.45 | 15.70 | 3 | Inactive |
| 15 | 2-$ClC_6H_4$ | $C_6H_{11}$ | 93 | 219–220 | $C_{14}H_{16}ClN_3S$ | 14.42 | 14.31 | 3 | Inactive |
| 16 | 2-$ClC_6H_4$ | $C_6H_5$ | 88 | 219–221 | $C_{14}H_{10}ClN_3S$ | 14.50 | 14.61 | 10 | Inactive |
| 17 | 3-$ClC_6H_4$ | $C_2H_5$ | 77 | 159–160 | $C_{10}H_{10}ClN_3S$ | 18.25 | 17.54 | 6 | Inactive |
| 18 | 3-$ClC_6H_4$ | iso-$C_4H_9$ | 71 | 138–139 | $C_{12}H_{14}ClN_3S$ | 15.56 | 15.70 | 3 | Inactive |
| 19 | 4-$ClC_6H_4$ | H | 66 | 286 | $C_8H_6ClN_3S$ | 19.62 | 19.86 | 12 | Inactive |
| 20 | 4-$ClC_6H_4$ | $C_2H_5$ | 93 | 203–204 | $C_{10}H_{10}ClN_3S$ | 17.42 | 17.54 | 6 | Inactive |
| 21 | 4-$ClC_6H_4$ | iso-$C_4H_9$ | 84 | 193–195 | $C_{12}H_{14}ClN_3S$ | 15.78 | 15.70 | 7 | Inactive |
| 22 | 4-$ClC_6H_4$ | $C_6H_{11}$ | 92 | 192–194 | $C_{14}H_{16}ClN_3S$ | 14.40 | 14.31 | 12 | Inactive |
| 23 | 2,4-$Cl_2C_6H_3$ | H | 76 | 273 dec. | $C_8H_5Cl_2N_3S$ | 17.33 | 17.07 | 7 | Active |
| 24 | 2-$OHC_6H_4$ | H | 79 | 290 | $C_8H_7N_3OS$ | 21.08 | 21.76 | 20 | Inactive |
| 25 | 2-$OHC_6H_4$ | $C_2H_5$ | 85 | 247–248 | $C_{10}H_{11}N_3OS$ | 19.29 | 19.00 | 3 | Inactive |
| 26 | 2-$OHC_6H_4$ | iso-$C_4H_9$ | 76 | 195–196 | $C_{12}H_{15}N_3OS$ | 16.84 | 16.87 | 20 | Inactive |
| 27 | 2-$OHC_6H_4$ | $C_6H_{11}$ | 89 | 171–172 | $C_{14}H_{17}N_3OS$ | 15.33 | 15.27 | 7 | Inactive |
| 28 | 2-$OHC_6H_4$ | 4′-$ClC_6H_4$ | 78 | 286–287 | $C_{14}H_{10}ClN_3OS$ | 13.97 | 13.83 | — | — |
| 29 | 3-$OHC_6H_4$ | $C_2H_5$ | 67 | 179–181 | $C_{10}H_{11}N_3OS$ | 18.88 | 19.00 | 6 | Inactive |
| 30 | 3-$OHC_6H_4$ | $CH_2CH{=}CH_2$ | 65 | 152–153 | $C_{11}H_{11}N_3OS$ | 18.30 | 18.02 | 3 | Inactive |
| 31 | 3-$OHC_6H_4$ | $n$-$C_3H_7$ | 73 | 187 | $C_{11}H_{13}N_3OS$ | 17.67 | 17.86 | 5 | Inactive |
| 32 | 3-$OHC_6H_4$ | iso-$C_3H_7$ | 76 | 212–213 | $C_{11}H_{13}N_3OS$ | 17.84 | 17.86 | 13 | Inactive |
| 33 | 3-$OHC_6H_4$ | $n$-$C_4H_9$ | 82 | 186–188 | $C_{12}H_{15}N_3OS$ | 16.62 | 16.87 | 5 | Active |
| 34 | 3-$OHC_6H_4$ | iso-$C_4H_9$ | 55 | 201–202 | $C_{12}H_{15}N_3OS$ | 16.64 | 16.87 | — | — |
| 35 | 3-$OHC_6H_4$ | $C_6H_{11}$ | 85 | 263–266 | $C_{11}H_{13}N_3OS$ | 15.04 | 15.27 | 3 | Active |
| 36 | 3-$OHC_6H_4$ | $C_6H_5$ | 83 | 247–248 | $C_{14}H_{11}N_3OS$ | 15.70 | 15.61 | 13 | Inactive |
| 37 | 4-$OHC_6H_4$ | $C_2H_5$ | 62 | 212–214 | $C_{10}H_{11}N_3OS$ | 19.25 | 19.00 | 20 | Inactive |
| 38 | 4-$OHC_6H_4$ | $CH_2CH{=}CH_2$ | 59 | 168–169 | $C_{11}H_{11}N_3OS$ | 17.63 | 18.02 | 7 | Inactive |
| 39 | 4-$OHC_6H_4$ | $n$-$C_3H_7$ | 77 | 178–181 | $C_{11}H_{13}N_3OS$ | 17.70 | 17.86 | 6 | Inactive |
| 40 | 4-$OHC_6H_4$ | iso-$C_3H_7$ | 81 | 285–286 | $C_{11}H_{13}N_3OS$ | 17.64 | 17.86 | 20 | Inactive |
| 41 | 4-$OHC_6H_4$ | $n$-$C_4H_9$ | 82 | 185–186 | $C_{12}H_{15}N_3OS$ | 16.96 | 16.87 | 14 | Inactive |
| 42 | 4-$OHC_6H_4$ | iso-$C_4H_9$ | 60 | 217–219 | $C_{12}H_{15}N_3OS$ | 17.13 | 16.87 | 13 | Inactive |
| 43 | 4-$OHC_6H_4$ | $C_6H_{11}$ | 85 | 247–249 | $C_{14}H_{17}N_3OS$ | 15.34 | 15.27 | 10 | Inactive |
| 44 | 4-$OHC_6H_4$ | $C_6H_5$ | 87 | 267–268 | $C_{14}H_{11}N_3OS$ | 15.35 | 15.61 | 10 | Inactive |
| 45 | 2-OH-5-$ClC_6H_3$ | $C_6H_5$ | 72 | 239–240 | $C_{14}H_{10}ClN_3OS$ | 14.08 | 13.84 | — | — |
| 46 | 2-OH-5-$BrC_6H_3$ | $C_2H_5$ | 61 | 205–206 | $C_{10}H_{10}BrN_3OS$ | 13.84 | 14.00 | 6 | Inactive |
| 47 | 2-OH-5-$BrC_6H_3$ | $CH_2CH{=}CH_2$ | 67 | 163–165 | $C_{11}H_{10}BrN_3OS$ | 13.41 | 13.46 | 3 | Inactive |
| 48 | 2-OH-5-$BrC_6H_3$ | iso-$C_3H_7$ | 57 | 200–204 | $C_{11}H_{12}BrN_3OS$ | 13.22 | 13.38 | 5 | Inactive |
| 49 | 2-OH-5-$BrC_6H_3$ | iso-$C_4H_9$ | 58 | 192–193 | $C_{12}H_{14}BrN_3OS$ | 12.48 | 12.80 | 7 | Inactive |
| 50 | 2-OH-5-$BrC_6H_3$ | $C_6H_{11}$ | 73 | 221–225 | $C_{14}H_{16}BrN_3OS$ | 11.98 | 11.87 | 12 | Inactive |
| 51 | 2-OH-5-$BrC_6H_3$ | $C_6H_5$ | 76 | 160–163 | $C_{14}H_{10}BrN_3OS$ | 11.98 | 12.07 | 6 | Inactive |
| 52 | 3,4,5-$(CH_3O)_3C_6H_2$ | H | 65 | 285 | $C_{11}H_{13}N_3O_3S$ | 15.45 | 15.73 | 4 | Inactive |
| 53 | 3,4,5-$(CH_3O)_3C_6H_2$ | $C_2H_5$ | 80 | 199–200 | $C_{13}H_{17}N_3O_3S$ | 14.04 | 14.23 | 7 | Active |
| 54 | 3,4,5-$(CH_3O)_3C_6H_2$ | iso-$C_4H_9$ | 77 | 204–206 | $C_{15}H_{21}N_3O_3S$ | 12.83 | 13.00 | 6 | Inactive |
| 55 | 3,4,5-$(CH_3O)_3C_6H_2$ | $C_6H_{11}$ | 89 | 228–230 | $C_{17}H_{23}N_3O_3S$ | 11.86 | 12.03 | 7 | Inactive |
| 56 | 3-Pyridyl | H | 57 | 295 dec. | $C_7H_6N_4S$ | 31.76 | 31.46 | 0.7 | Inactive |
| 57 | 3-Pyridyl | $C_2H_5$ | 84 | 177–178 | $C_9H_{10}N_4S$ | 26.76 | 27.18 | 6 | Active |
| 58 | 3-Pyridyl | iso-$C_4H_9$ | 92 | 202–203 | $C_{11}H_{14}N_4S$ | 24.34 | 23.93 | 6 | Inactive |
| 59 | 3-Pyridyl | $C_6H_{11}$ | 95 | 224–226 | $C_{13}H_{16}N_4S$ | 21.22 | 21.54 | 6 | Inactive |
| 60 | 4-Pyridyl | H | 80 | 290 | $C_7H_6N_4S$ | 30.93 | 31.46 | 7 | Inactive |
| 61 | 4-Pyridyl | $C_2H_5$ | 89 | 231–233 | $C_9H_{10}N_4S$ | 26.70 | 27.18 | 12 | Inactive |
| 62 | 4-Pyridyl | iso-$C_4H_9$ | 83 | 238–239 | $C_{11}H_{14}N_4S$ | 23.66 | 23.93 | 6 | Inactive |
| 63 | 4-Pyridyl | $C_6H_{11}$ | 90 | 298 dec. | $C_{13}H_{16}N_4S$ | 21.12 | 21.54 | 8 | Inactive |

for further studies on electrolyte excretion. The results are presented in Table IV.

**Results and Structure-Activity Relationship**—Among the 63 compounds screened 14 were found to possess diuretic activity.

The introduction of a phenyl group in Position 3 of the triazole ring gave the most active compounds in the present series. The introduction of substituents in this phenyl (except chlorine in ortho position) or the replacement of the phenyl with pyridyl group reduced the activity considerably. Substitution in Position 4 did not significantly alter the activity.

3-Phenyl-4-allyl-5-mercapto-1,2,4(H)-triazole (2) and 3-*o*-chlorophenyl-4-allyl-5-mercapto-1,2,4(H)-triazole (10) were the most active compounds in the present series.

## EXPERIMENTAL

**3-(3′,4′,5′-Trimethoxyphenyl)-4-ethyl-5-mercapto-1,2,4(H)-triazole (53)**—1-(3′,4′,5′-Trimethoxybenzoyl)-4-ethylthiosemicarbazide (4.7 g., 0.015 mole) was dissolved in 1 *N* sodium hydroxide

**Table II**—1,4 Disubstituted Thiosemicarbazides

R—CO—NH—NH—CS—NH—R′

| Compd. No. | R | R′ | Yield, % | M.p., °C. | Molecular formula | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|
| 1 | $C_6H_5$ | $C_2H_5$ | 94 | 192–193 | $C_{10}H_{13}N_3OS$ | 18.83 | 18.91 |
| 2 | $C_6H_5$ | $CH_2$—CH=$CH_2$ | 89 | 172–173 | $C_{11}H_{13}N_3OS$ | 17.87 | 17.62 |
| 3 | $C_6H_5$ | *n*-$C_3H_7$ | 76 | 163–164 | $C_{11}H_{15}N_3OS$ | 17.72 | 17.65 |
| 4 | $C_6H_5$ | *n*-$C_4H_9$ | 83 | 154–155 | $C_{12}H_{17}N_3OS$ | 16.73 | 16.48 |
| 5 | $C_6H_5$ | iso-$C_4H_9$ | 80 | 177–178 | $C_{12}H_{17}N_3OS$ | 16.73 | 16.68 |
| 6 | $C_6H_5$ | $C_6H_{11}$ | 80 | 170–172 | $C_{14}H_{19}N_3OS$ | 15.16 | 14.80 |
| 7 | 2-$ClC_6H_4$ | $C_2H_5$ | 91 | 163–164 | $C_{10}H_{12}ClN_3OS$ | 16.31 | 15.90 |
| 8 | 2-$ClC_6H_4$ | $CH_2$—CH=$CH_2$ | 87 | 165 | $C_{11}H_{12}ClN_3OS$ | 15.58 | 15.38 |
| 9 | 2-$ClC_6H_4$ | *n*-$C_3H_7$ | 79 | 161–162 | $C_{11}H_{14}ClN_3OS$ | 15.47 | 15.65 |
| 10 | 2-$ClC_6H_4$ | iso-$C_3H_7$ | 80 | 157–158 | $C_{11}H_{14}ClN_3OS$ | 15.47 | 15.21 |
| 11 | 2-$ClC_6H_4$ | *n*-$C_4H_9$ | 86 | 131–132 | $C_{12}H_{16}ClN_3OS$ | 14.71 | 14.53 |
| 12 | 2-$ClC_6H_4$ | iso-$C_4H_9$ | 73 | 161–162 | $C_{12}H_{16}ClN_3OS$ | 14.71 | 14.66 |
| 13 | 2-$ClC_6H_4$ | $C_6H_{11}$ | 82 | 161–162 | $C_{14}H_{18}ClN_3OS$ | 13.48 | 13.35 |
| 14 | 2-$ClC_6H_4$ | $C_6H_5$ | 64 | 139–141 | $C_{14}H_{12}ClN_3OS$ | 13.74 | 14.04 |
| 15 | 3-$ClC_6H_4$ | $C_2H_5$ | 87 | 185–186 | $C_{10}H_{12}ClN_3OS$ | 16.31 | 16.42 |
| 16 | 3-$ClC_6H_4$ | iso-$C_4H_9$ | 61 | 195–196 | $C_{12}H_{16}ClN_3OS$ | 14.71 | 14.18 |
| 17 | 4-$ClC_6H_4$ | $C_2H_5$ | 89 | 203–204 | $C_{10}H_{12}ClN_3OS$ | 16.31 | 16.50 |
| 18 | 4-$ClC_6H_4$ | iso-$C_4H_9$ | 72 | 193–194 | $C_{12}H_{16}ClN_3OS$ | 14.71 | 14.43 |
| 19 | 4-$ClC_6H_4$ | $C_6H_{11}$ | 85 | 215–216 | $C_{14}H_{18}ClN_3OS$ | 13.48 | 13.85 |
| 20 | 2,4-$Cl_2C_6H_3$ | H | 87 | 213–214 | $C_8H_7Cl_2N_3OS$ | 15.91 | 16.08 |
| 21 | 2-$OHC_6H_4$ | $C_2H_5$ | 86 | 242–244 | $C_{10}H_{13}N_3O_2S$ | 17.57 | 17.77 |
| 22 | 2-$OHC_6H_4$ | iso-$C_4H_9$ | 66 | 164–166 | $C_{12}H_{17}N_3O_2S$ | 15.73 | 15.51 |
| 23 | 2-$OHC_6H_4$ | $C_6H_{11}$ | 72 | 192–194 | $C_{14}H_{19}N_3O_2S$ | 14.33 | 14.14 |
| 24 | 2-$OHC_4H_4$ | 4-$ClC_6H_4$ | 78 | 199–201 | $C_{14}H_{12}ClN_3O_2S$ | 13.06 | 13.52 |
| 25 | 3-$OHC_6H_4$ | $C_2H_5$ | 73 | 209–210 | $C_{10}H_{13}N_3O_2S$ | 17.57 | 17.48 |
| 26 | 3-$OHC_6H_4$ | $CH_2CH$=$CH_2$ | 75 | 208 | $C_{11}H_{13}N_3O_2S$ | 16.73 | 16.87 |
| 27 | 3-$OHC_6H_4$ | *n*-$C_3H_7$ | 69 | 204–205 | $C_{11}H_{15}N_3O_2S$ | 16.60 | 16.32 |
| 28 | 3-$OHC_6H_4$ | iso-$C_3H_7$ | 69 | 211–212 | $C_{11}H_{15}N_3O_2S$ | 16.60 | 16.46 |
| 29 | 3-$OHC_6H_4$ | *n*-$C_4H_9$ | 71 | 193–194 | $C_{12}H_{17}N_3O_2S$ | 15.73 | 15.21 |
| 30 | 3-$OHC_6H_4$ | iso-$C_4H_9$ | 68 | 201–202 | $C_{12}H_{17}N_3O_2S$ | 15.73 | 15.91 |
| 31 | 3-$OHC_6H_4$ | $C_6H_{11}$ | 75 | 214–215 | $C_{14}H_{19}N_3O_2S$ | 14.33 | 13.96 |
| 32 | 3-$OHC_6H_4$ | $C_6H_5$ | 65 | 202–203 | $C_{14}H_{13}N_3O_2S$ | 14.63 | 14.67 |
| 33 | 4-$OHC_6H_4$ | $C_2H_5$ | 82 | 204–205 | $C_{10}H_{13}N_3O_2S$ | 17.57 | 17.20 |
| 34 | 4-$OHC_6H_4$ | *n*-$C_3H_7$ | 85 | 188–189 | $C_{11}H_{15}N_3O_2S$ | 16.60 | 16.33 |
| 35 | 4-$OHC_6H_4$ | iso-$C_4H_9$ | 80 | 190–191 | $C_{12}H_{17}N_3O_2S$ | 15.73 | 15.80 |
| 36 | 4-$OHC_6H_4$ | $C_6H_{11}$ | 84 | 203–204 | $C_{14}H_{19}N_3O_2S$ | 14.33 | 14.42 |
| 37 | 4-$OHC_6H_4$ | $C_6H_5$ | 73 | 185–186 | $C_{14}H_{13}N_3O_2S$ | 14.63 | 15.05 |
| 38 | 2-OH-5-$ClC_6H_3$ | $C_6H_5$ | 83 | 175–176 | $C_{14}H_{12}ClN_3O_2S$ | 13.06 | 13.32 |
| 39 | 2-OH-5-$BrC_6H_3$ | $C_2H_5$ | 78 | 209–210 | $C_{10}H_{12}BrN_3O_2S$ | 13.20 | 13.24 |
| 40 | 2-OH-5-$BrC_6H_3$ | iso-$C_4H_9$ | 68 | 212–214 | $C_{12}H_{16}BrN_3O_2S$ | 12.13 | 12.26 |
| 41 | 2-OH-5-$BrC_6H_3$ | $C_6H_{11}$ | 78 | 200–202 | $C_{14}H_{18}BrN_3O_2S$ | 11.29 | 11.11 |
| 42 | 2-OH-5-$BrC_6H_3$ | $C_6H_5$ | 88 | 189–190 | $C_{14}H_{12}BrN_3O_2S$ | 11.47 | 11.74 |
| 43 | 3,4,5-$(CH_3O)_3C_6H_2$ | $C_2H_5$ | 74 | 197–199 | $C_{13}H_{19}N_3O_4S$ | 13.41 | 13.02 |
| 44 | 3,4,5-$(CH_3O)_3C_6H_2$ | iso-$C_4H_9$ | 67 | 186–187 | $C_{15}H_{23}N_3O_4S$ | 12.31 | 12.03 |
| 45 | 3,4,5-$(CH_3O)_3C_6H_2$ | $C_6H_{11}$ | 83 | 167–168 | $C_{17}H_{25}N_3O_4S$ | 11.44 | 11.25 |
| 46 | 3-Pyridyl | $C_2H_5$ | 58 | 163–165 | $C_9H_{12}N_4OS$ | 25.00 | 25.25 |
| 47 | 3-Pyridyl | iso-$C_4H_9$ | 68 | 173–175 | $C_{11}H_{16}N_4OS$ | 22.22 | 22.07 |
| 48 | 3-Pyridyl | $C_6H_{11}$ | 87 | 190–192 | $C_{13}H_{18}N_4OS$ | 20.15 | 20.17 |
| 49 | 4-Pyridyl | $C_2H_5$ | 74 | 230–231 | $C_9H_{12}N_4OS$ | 25.00 | 24.51 |
| 50 | 4-Pyridyl | iso-$C_4H_9$ | 71 | 207–208 | $C_{11}H_{16}N_4OS$ | 22.22 | 23.34 |
| 51 | 4-Pyridyl | $C_6H_{11}$ | 86 | 218–219 | $C_{13}H_{18}N_4OS$ | 20.15 | 20.51 |

(25 ml.) and the solution was heated under reflux for 1 hr. At the end of this period, the reaction mixture was filtered, the filtrate was cooled and acidified with hydrochloric acid. The white solid that separated was filtered after 30 min., washed with water, and dried to give 3-(3′,4′,5′-trimethoxyphenyl)-4-ethyl-5-mercapto-1,2,4(H)-triazole; (3.56 g., 80%), m.p. 199–200°.

**3-Phenyl-4-cyclohexyl-5-mercapto-1,2,4(H)-triazole (7)**—To 1-benzoyl-4-cyclohexylthiosemicarbazide (1 g., 0.0036 mole) was added 5% aqueous sodium carbonate (25 ml.) and the mixture refluxed for 4 hr. It was then worked up as described above to give 3-phenyl-4-cyclohexyl-5-mercapto-1,2,4(H)-triazole (0.792 g., 84%) and crystallized from ethanol–water (1:1), m.p. 193°.

**1-Salicyloyl-6-cyclohexylthiosemicarbazide (23)**—To a solution of 2-hydroxybenzhydrazide (3.04 g., 0.02 mole) in ethanol (20 ml.) was added cyclohexylisothiocyanate (2.85 ml., 0.02 mole), followed by aqueous sodium hydroxide (10 ml. of 2 *N*, 0.02 mole). This reaction mixture was stirred for 3 hr. at room temperature and was left for 36 hr. It was then filtered and the filtrate acidified with hydrochloric acid. The white solid that precipitated was collected, washed with water, and dried to get 1-salicyloyl-4-phenylthiosemicarbazide (4.22 g., 72%) which on crystallization from 70% ethanol melted at 192–194°.

**3-Phenyl-4-n-butyl-5-mercapto-1,2,4-(H)-triazole (5)**—Benzhydrazide (2.72 g., 0.02 mole) was dissolved in ethanol (25 ml.) and to this soltuion was added *n*-butylisothiocyanate (2.3 g., 0.02 mole) followed by sodium hydroxide solution (25 ml. of 2 *N*). The mixture was heated to reflux on a water bath for 5 hr. and then poured into cold water. On acidification with hydrochloric acid 3-phenyl-4-*n*-butyl-5-mercapto-1,2,4(H)-triazole separated (2.31 g., 50%). On crystallization from ethanol the product melted at 130–131°. The mixed melting point with the product obtained on cyclization of

**Table III**—Criteria for Judging Diuretic Activity

| Cages | Cumulative Urine Volume Required for Acceptable Activity in ml. | Cumulative Urine Volume Required for Rejection in ml. |
|---|---|---|
| I | 19.3 or more | 3.7 or less |
| I + II | 30.8 or more | 15.2 or less |
| I + II + III | 42.3 or more | 26.7 or less |
| I + II + III + IV | 53.7 or more | 38.1 or less |

Table IV—Diuretic Effect after Administration of the Compounds Orally to Rats; Values in Urine as % of Control

| Compound No. | Volume | $Na^+$ | $K^+$ | $Cl^-$ |
|---|---|---|---|---|
| 1 | 125 | 121 | 219 | 277 |
| 2 | 163.5 | 772.5 | 118 | 378.5 |
| 4 | 125.5 | 182 | 141.5 | 296 |
| 5 | 152.5 | 138 | 280 | 287 |
| 6 | 141.5 | 520.0 | 162.5 | 323.0 |
| 7 | 133.5 | 90 | 158 | 266 |
| 8 | 154.5 | 276.0 | 164 | 255 |
| 10 | 155.8 | 1000 | 139.3 | 410 |
| Hydrochloro-thiazide | 166 | 1000 | 250 | 538.5 |

1-phenyl-4-*n*-butylthiosemicarbazide was not depressed.

**3-p-Chlorophenyl-6-isopropyl-1,2,4(H)-triazole-5-sulfonamide**—A stirred mixture of 3-*p*-chlorophenyl-4-isopropyl-5-mercapto-1,2,4-(H)-triazole (5.0 g.), water (135 ml.), and ferric chloride solution (0.7 ml. of 60%) was stirred and cooled to 0°. Chlorine gas was then passed into the mixture for 1 hr. maintaining the temperature between 0–5°. The reaction mixture was allowed to stand at this temperature for 15 min. more and then filtered. The solid was pressed on filter paper and immediately added to aqueous ammonia (150 ml. of 20%). This solution was left at room temperature for 6 hr. and then filtered. The filtrate was acidified with hydrochloric acid to pH 6. The white solid that separated, on purification by redissolving in alkali and precipitating with acid followed by crystallization from ethanol, gave 3-*p*-chlorophenyl-4-isopropyl-1,2-4(H)-triazole-5-sulfonamide (2.8 g.), m.p. 214–215°.

*Anal.*—Calcd. for $C_{11}H_{13}ClN_4O_2S$: N, 18.64. Found: 18.69.

Similarly, 3-*o*-chlorophenyl-4-phenyl-1,2,4(H)-triazole-5-sulfonamide was obtained in 32% yield, m.p. 240°.

*Anal.*—Calcd. for $C_{14}H_{11}ClN_4O_2S$: N, 16.74. Found: N, 16.53.

## REFERENCES


(1) M. H. Shah, M. Y. Mhasalkar, and V. M. Patki, *J. Sci. Ind. Res. (India)*, **19 C**, 67(1960).

(2) H. L. Yale and J. J. Piala, *J. Med. Chem.*, **9**, 42(1966).

(3) I. B. Douglass and T. B. Johnson, *J. Am. Chem. Soc.*, **60**, 1486(1938).

(4) R. O. Roblin, Jr. and J. W. Clapp, *ibid.*, **72**, 4890(1950).

(5) K. N. Modi, M. N. Vartak, N. N. Shah, K. Lotlikar, and U. K. Sheth, *Arch. Intern. Pharmacodyn.*, **144**, 51(1963).

(6) K. N. Modi, N. N. Shah, and U. K. Sheth, *ibid.*, **144**, 61 (1963).


## ACKNOWLEDGMENTS AND ADDRESSES


Received November 8, 1968 from the *Department of Chemotherapy, Haffkine Institute, Bombay 12, and the Departent of Pharmacology, Sheth G. S. Medical College, Bombay, India (U.K.S.).*

Accepted for publication March 18, 1969.



* To whom communications regarding this paper should be addressed.



The authors thank Dr. N. K. Dutta, Director, Haffkine Institute for providing facilities for this work, and to Shri M. T. Jaokar and his colleagues for the microanalyses.


# *DRUG STANDARDS*

# Analysis of Metronidazole


**MURRAY M. TUCKERMAN and TATJANA BIĆAN-FIŠTER***

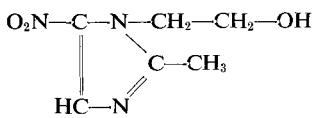


**Abstract** □ The literature on the identification, assay, and use of metronidazole has been surveyed. Based on published information, private communications, and laboratory experimentation, qualitative tests and quantitative assays have been developed for metronidazole, metronidazole suppositories (vaginal tablets), and metronidazole tablets. Extraction of metronidazole from suppositories and tablets is with hot acetone. Assays are based on titration of metronidazole in acetic anhydride with 0.1 *N* perchloric acid in glacial acetic acid, using malachite green indicator. The visual endpoint coincides with that determined potentiometrically. Supporting data is presented, including UV and IR spectra.



**Keyphrases** □ Metronidazole dosage forms—analysis □ Colorimetric method—identity □ UV spectrophotometry—identity □ IR spectrophotometry—identity


Metronidazole,[1] $C_6H_9N_3O_3$; mol. wt. 171.15, is 2-methyl - 5 - nitroimidazole - 1 - ethanol or 1 - (2 - hydroxyethyl)-2-methyl-5 nitroimidazole. It was recognized in "Addendum 1964" of the BP 1963 (1) as the drug and in the form of tablets. These two forms and the suppository have been admitted to USP XVIII. The structural formula may be represented as



## EXPERIMENTAL

**Physical Properties**—Metronidazole occurs as white to pale yellow crystals or crystalline powder, stable in air, but darkening on exposure to light. It is sparingly soluble in water, in alcohol, and in chloroform, and is slightly soluble in ether. The melting range is 159–163°.

**Identity Tests**—*A.*—Heat about 10 mg. in a water bath for 5 min. with 1 ml. of water, 0.25 ml. of hydrochloric acid, and 10 mg. of zinc powder, filter, cool, add 1 ml. of freshly prepared sodium nitrite solution (1 in 100), then remove excess nitrite by addition of 1 ml. of freshly prepared sulfamic acid solution (1 in 100). To 1 ml. of this solution add 1 ml. of betanaphthol T.S.: an intense red color is produced. (This differs from the BP test in that the reaction takes place in an acid medium.)

[1] Flagyl, G. D. Searle & Co., Chicago, Ill.