addition of alkaline ascorbate solution the reaction is extremely photosensitive. These investigators avoided erratic results by placing the tubes of samples inside a box which contains two General Electric 20-watt ultraviolet black light bulbs. They found that rigid control of the lighting conditions enhanced the intensity of fluorescence and improved reproducibility. This finding further supports the contention that light is an essential component in the spectrophotofluorometric assay of norepinephrine during the THI reaction.

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Cyclized Substituted Thioureas III

1-Substituted-tetrazolines-5-thiones

By RONALD E. ORTH

A convenient synthesis and purification of some 1-substituted-tetrazoline-5-thiones is accomplished. Decreased per cent yields with increased chain length lends some credence to the probability that steric hindrance occurs in the ring closing step. The infrared studies show identifying bands for —N=C=S, C=S, cyclic —N—N=N—, and the tetrazole ring. Low toxicity for these compounds is indicated.

QUALIFIED success with propylthiouracil (1) and methimazole (2) during hyperthyroid therapy has led to the synthesis of chemically related compounds and to their subsequent *in vitro* and *in vivo* trials. Countless antibacterial and tuberculostatic screening programs have also been carried out using compounds which embody the thiourea moiety.

Some 1-alkyltetrazoline-5-thiones were prepared by synthesizing the methyl ester of *N*-alkyldithiocarbamic acid and refluxing it with sodium azide (3). A more convenient method consists of preparing the sodium *N*-alkyl dithiocarbamate from carbon disulfide, the corresponding amine, and sodium hydroxide. Ethyl chloroformate and the dithiocarbamate give ethyl *N*-alkyldithiocarbamate, which upon decomposition yields carbonyl sulfide, ethanol, and the desired alkyl isothiocyanate (R—N=C=S). The 1-substituted-tetrazoline-5-thiones were isolated following reflux of the isothiocyanate with sodium azide and acidification. The lower yields with increased chain length suggests that there is a steric effect since it has been demonstrated that 1-aryl substituents increases the yields appreciably (4).

Tetrazole and its substitution products usually show only a small amount of end absorption in the ultraviolet region of the spectrum (240–250 mμ) (5). Otting found that tetrazole gave large peaks at 1520, 1270, 1160, 1100, 1020, 915, and 670 cm.$^{-1}$ and a number of characteristic small peaks (6). Lieber, *et al.*, found some 5-substituted tetrazoles to give results interpreted as tetrazole ring absorption bands in the 8.9 to 10 μ region (7). In later work with 1-aryl-tetrazoline-5-thiones more definite tetrazole skeletal assignments were demonstrated. The bands were found at approximately 1100, 1080, 1045, 1020, and 990 cm.$^{-1}$ Frequencies were also assigned for N—C=S, C=S, and cyclic —N—N=N— linkages (8). The spectra for the 1-substituted-tetrazoline-5-thiones summarized in Table I closely approximate the Lieber assigned values and support his hypothesis showing the presence of sulfur in the thione form. Characteristic sulfhydryl bands were not seen for any of the compounds tested from this series. The results of this investigation emphasize the uniformity of the data obtained using 1-aryl and 1-alkyl substituents in the tetrazoline-5-thione system.

LD_{50}'s of 207 ± 27 mg./Kg. and 215 ± 23 mg./Kg. body weight were obtained for 1-methyl- and 1-isopropyltetrazoline-5-thiones, respectively, using five groups per compound, each group having 20 young male white mice.

EXPERIMENTAL

1-Substituted-Tetrazoline-5-thiones.—Three-tenths mole (19.5 Gm.) of sodium azide is dissolved in 300 ml. of water and filtered into a 500 ml. round-bottom flask and two-tenths mole of an isothiocyanate is added, followed by refluxing. Cease the operation and remove any unreacted isothiocyanate by ether extraction when the green reaction mixture turns colorless. Acidify the aqueous solution to a pH of 3 with HCl, filter, and extract the filtrate with ether. Wash the ether solution with small portions of ice water, dry over anhydrous sodium sulfate, and decolorize with activated charcoal. The dried acidic ether extract is evaporated to a yellow-brown oil, which in turn forms pale yellow crystals, using the Rinco rotary evaporator and steam bath. These crystals are blotted dry on filter paper, and recrystallized from 100–115° petroleum ether.

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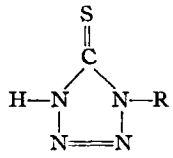
TABLE I.—1-SUBSTITUTED-TETRAZOLINE-5-THIONES



R	M.p. °C	Yield,[a] %	Nitrogen, % Calcd.	Nitrogen, % Obs.[b]	Infrared Spectra[c, e, f] —N—C=S	C=S	Cyclic —N—N=N	Tetrazole Skeletal Bands			
Methyl	125–26	52	48.2	48.5	1510(m)	1350(m)	1300(m)		1066(w)	1042(s)	
Ethyl	50	37	...	...	1500(w)		1275(s)	1088(s)		1045(s)	983(m)
n-Propyl	77–78	24	38.8	40.0	1510(s)	1350(s)	1290(m)	1112(m)		1050(s)	995(s)
iso-Propyl[d]	89–90	34	38.8	38.7							
Allyl	153	22	39.5	39.5	1500(s)	1350(m)	1304(m)		1075(m)	1053(s)	990(s)
iso-Butyl[d]	64–65	25	35.4	35.4							
t-Butyl[d]	98–99	29	35.4	35.4							
Benzyl	148	87	31.1	31.9	1495(m)		1290(w)	1090(w)		1053(m)	

a Average yield based on two runs per compound. *b* Weiler and Strauss, Microanalytical laboratory, Oxford, England (average of two runs). *c* Dr. Paul R. Caudill, College of Agriculture, University of Kentucky, Lexington. *d* Not subjected to infrared studies. *e* Perkin Elmer 21 instrument used; Nujol phase; filter out; NaCl prism; 927 program; speed 1 μ/min.: response 1; suppression 0. *f* Key: (s) = strong band; (m) = medium band; (w) = weak band.

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Determination of Ergotamine and Ergotaminine in Pharmaceutical Preparations

By THOMAS G. ALEXANDER

Ergotamine and its diastereoisomer, ergotaminine, are separated from each other and from other ingredients by column chromatography. The isolated fractions are then assayed by the classical van Urk method. Identity and purity of the fractions are confirmed by paper chromatography. A number of commercial products were analyzed successfully.

ERGOTAMINE in solution forms an equilibrium mixture of the diastereoisomers, ergotamine, and ergotaminine. This study was undertaken to develop a practical quantitative method for the assay of both ergotamine and ergotaminine in injection solutions. Of the different techniques that have been described for this purpose, column chromatography appeared to be the best suited. Larger amounts of alkaloid can be recovered more efficiently on a column than with methods involving the extraction of spots from paper or thin-layer chromatograms (1–3). Yet large samples are not required as with the polarimetric methods (4). Also a column chromatographic technique does not require the use of as much time and specialized glassware as would one involving countercurrent extraction (5).

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The method presented involves partition chromatography using a column prepared by adsorbing 1:4 citric acid solution on siliceous earth. This column extracts ergotamine from a chloroform solution containing both ergotamine and ergotaminine. The latter passes through in the effluent and is assayed. Ergotamine is recovered by chloroform extraction of the extruded column. This procedure was submitted to the Committee on Revision of the "United States Pharmacopeia" and it has been incorporated into the Monograph for Ergotamine Tartrate Injection. The procedural details are contained in this revised monograph (6).

To analyze ergotamine salts and simple tablet mixtures, weighed portions are dissolved in or triturated with 1:100 tartaric acid solution; the sample is then analyzed in the manner described in the revised monograph for injections.

EXPERIMENTAL AND DISCUSSION

In following the methods described by Berg (7) and van de Langerijt (8) involving the use of benzene as eluent, both ergotamine and ergotaminine rapidly epimerized and deteriorated. Carless (9)