

and standardised as described by Ayres and Wells<sup>4</sup>. Working solutions were prepared as needed by the dilution of the stock solution. A 0.2% of aqueous solution of TH was used. All other solutions were prepared from reagent grade chemicals. Beckman Model DB spectrophotometer with matched 1 cm silica cells was used for absorbance measurements.

**Procedure.**—An aliquot of the sample solution containing 2.5–72 ppm of osmium was transferred to a 25 ml volumetric flask. 5 ml of 5 M hydrochloric acid and 5 ml of 0.2% TH solution were added and diluted to the mark with doubly distilled water. The solution was thoroughly mixed and the absorbance was measured after 10 minutes at 640 nm against a reagent blank prepared in the same way. The osmium concentration of the sample was calculated by reference to a previously prepared calibration curve.

#### Results and Discussion

Osmium (VIII) oxidises TH to a blue-coloured product which is thought to be a free radical<sup>3</sup>. The sensitivity and the stability of the blue-coloured product depend on the nature and strength of acid medium. The sensitivity of the reaction in four acid media (1.0 M) is in the order, HCl > H<sub>2</sub>SO<sub>4</sub> > H<sub>3</sub>PO<sub>4</sub> > HAC. The stability of the blue colour in 1.0 M HCl, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub> and HAC is 25, 10, 5 and 4 minutes respectively. Hence hydrochloric acid medium is preferred. The maximum colour intensity is observed in 0.2–1.5 M HCl. Therefore 1.0 M hydrochloric acid is used in these studies.

The electronic spectrum of the blue-coloured species shows the maximum absorption around 640 nm with a shoulder at 490 nm. The reagent does not absorb at these wavelengths. A thirty-fold molar excess of TH over osmium is required for complete colour development. The constant absorbance values are obtained 5 minutes after adding the reagent to the osmium solution. The order of addition of reagents has no effect on the absorbance values which are insensitive to temperature in the range 5–40° C.

Beer's law is valid within the concentration range of 0.1 to 2.8 ppm of osmium and the optimum range is 1.0 to 2.4 ppm of osmium. At 640 nm, the molar absorptivity is  $1.98 \times 10^4$  litre mole<sup>-1</sup> cm<sup>-1</sup>. For log I<sub>0</sub>/I = 0.001, the sensitivity of the reaction is 0.0096 µg Os cm<sup>-2</sup>.

In order to assess the possible analytical applications of the reaction, the effect of diverse ions was studied. The following amounts (µg/ml) of foreign ions were found to give less than 2% error in the determination of 2 ppm of osmium;

Pt (IV) 15; Rh (III) 12; Ru (III) 1; Ir (III) 15; Co (II) 100; Ni (II) 150; UO<sub>2</sub> (II) 100; fluoride 1100; chloride 1800; bromide 800; nitrate 900; sulphate 1000; phosphate 300; oxalate 850; EDTA 10. Iodide, thiosulphate, Pd (II), Au (III), Fe (III), Ag (I), Ce (IV) and V (V) interfere seriously. Attempts to increase the tolerance limits of some cations were unsuccessful.

**Determination of osmium in ores.**—Synthetic mixtures containing platinum metals corresponding to osmiridium or syserkite were prepared and the osmium content was determined. The results are given in Table I.

TABLE I  
Determination of osmium in synthetic mixtures corresponding to osmiridium or syserkite

Osmium present ppm	Rh, ppm	Ir, ppm	Ru, ppm	Pd ppm	Pt, ppm	Osmium found, ppm
1.00	..	0.250	0.150	..	0.050	1.02
1.50	..	0.425	0.225	..	0.075	1.56
2.00	..	0.500	0.300	..	0.100	2.05

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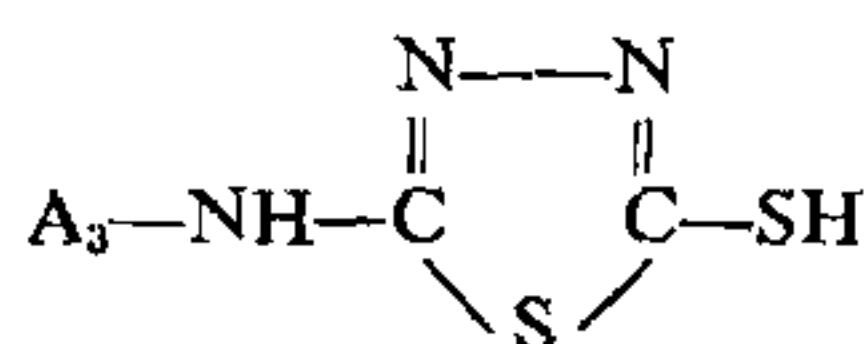
#### STUDIES IN THIADIAZOLES: SYNTHESIS OF SOME 2-SUBSTITUTED-5-MERCAPTO-1, 3, 4-THIADIAZOLES

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4-ARYLTHIOSEMICARBAZIDES (Ar NH-C-NHNH<sub>2</sub>) on cyclisation yield 2-arylamino-5-mercapto-1, 3, 4-thiadiazoles which have been found to be biologically active<sup>1</sup>. The SH group is an important toxophore as —OH especially to fungi<sup>2</sup> and when a heterocyclic compound contains an —SH group adjacent to a nitrogen atom, useful fungitoxic compounds are often obtained because of the possibility of chelation<sup>2,3</sup>. The 2-arylamino-5-mercapto-1, 3, 4-thiadiazoles fulfil these requirements and therefore a study of these compounds was undertaken with a view to studying their fungicidal activity.

In the present communication six thiadiazoles have been prepared by the reaction of 4-arylthiosemicarbazides with carbon disulphide and diethylformamide<sup>4</sup>. (i) 4-Arylthiosemicarbazides were prepared

TABLE I



Sl. No.	Ar.	Yield	M.P. °C	Mol. formula	% Sulphur		Dia. of zone of inh. in mm
					Found	Reqd.	
1.	4-Iodo-2-methylphenyl	60	184	C <sub>9</sub> H <sub>8</sub> N <sub>3</sub> S <sub>2</sub> I <sub>2</sub>	18.46	18.34	..
2.	2, 5-Dimethoxyphenyl	70	204	C <sub>10</sub> H <sub>11</sub> O <sub>2</sub> N <sub>3</sub> S <sub>2</sub>	23.66	23.79	10
3.	2, 5-Dichlorophenyl	65	130	C <sub>8</sub> H <sub>5</sub> N <sub>3</sub> S <sub>2</sub> Cl <sub>2</sub>	23.16	23.02	12
4.	2-Bromo-4-methylphenyl	68	186	C <sub>9</sub> H <sub>8</sub> N <sub>3</sub> S <sub>2</sub> Br	21.32	21.19	..
5.	4-Chloro-2-methoxyphenyl	62	172	C <sub>9</sub> H <sub>8</sub> N <sub>3</sub> OS <sub>2</sub> Cl	23.58	23.40	11
6.	4-Chloro-2-methylphenyl Benzoic acid	64	182	C <sub>9</sub> H <sub>8</sub> N <sub>3</sub> S <sub>2</sub> Cl	24.72	24.85	10 13

by the method of Kazokov<sup>5</sup>. (ii) 2-Arylamino-5-mercapto-1, 3, 4-thiadiazoles: A mixture of appropriate 4-arylthiosemicarbazide (0.01 M), carbon disulphide (0.01 M) and dimethylformamide (20 ml) was refluxed on the water bath for about 1-1.5 hours at 65-70° C. The excess of the solvent was distilled off under reduced pressure. On cooling, the product separated out and was purified.

The details of the compounds thus prepared are listed in Table I.

**Pharmacology:** The prepared thiadiazoles were tested against five strains of fungi, viz., *C. albicans*, *C. utilis*, *A. tenuis*, *A. niger* and *A. flavus* for fungicidal activity at a concentration of 150 ± 10 µg/ml in 50% ethanol. The strength is reported by measuring the diameter of zone of inhibition of a particular microorganism (Table I) and the results were compared against benzoic acid.

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#### GENETICS AND BIOCHEMISTRY OF PYRIDOXINE REQUIRING MUTANTS OF *ASPERGILLUS NIDULANS*

IN our attempts to isolate a number of nutritionally deficient mutant strains of *Aspergillus nidulans* by ultraviolet irradiation of spores from a parent strain *y; ribo<sub>1</sub>* (spore colour yellow and requiring riboflavin for growth), eight pyridoxine requiring mutants were obtained. Seven of them were found to be non-allelic by complementation tests<sup>1</sup>. The mutants were outcrossed with a strain *bi; w<sub>3</sub>, ade<sub>3</sub>* (white spore colour requiring both biotin and adenine for growth). Strains requiring only pyridoxine for growth were isolated from the perithecia and designated as *pyro<sub>5</sub>*, *pyro<sub>6</sub>*, *pyro<sub>7</sub>*, *pyro<sub>8</sub>*, *pyro<sub>9</sub>*, *pyro<sub>10</sub>* and *pyro<sub>12</sub>*, with the spore colours white, yellow which are recessive and the dominant green. Since all the mutant strains were available with white spore colours, excepting *pyro<sub>8</sub>*, strains with white conidial colours were taken up for the present study, along with *pyro<sub>8</sub>* with yellow conidial colour.

The pyridoxineless mutants were allowed to grow at 23° C, 30° C, 37° C and 42° C on pyridoxine supplemented Minimal Medium<sup>1</sup> and were found to be temperature independent. The growth promoting effect of sugar phosphates, three carbon compounds of the glycolytic pathway, purines, pyrimidines, nicotinic acid and some other alleged precursors of pyridoxine are presented in Table I. From the results presented, it can be seen that β-glycerophosphate, dihydroxy acetone phosphate, 2- and 3-phosphoglycerates could promote the growth of mutant strain *w<sub>3</sub>; pyro<sub>9</sub>*. The mutant

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