SPECTROPHOTOMETRIC DETERMINATION OF ISONIAZID

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PYRIDINE-4-CARBOXYLIC acid hydrazide commercially known as isoniazid (INH) is an antitubercular drug. The importance of INH as an antitubercular drug has prompted many investigators to work out methods for its rapid determination. Many proposed colorimetric methods[1–3] which are unsatisfactory for one reason or another. The proposed method is based on the formation of amber coloured complex of INH with vanadophoshoric acid in acetic acid medium. The method offers the advantages of sensitivity, simplicity and rapidity without the need for extraction or heating.

Experimental

Reagents

A stock solution of INH (U.S.P. grade) in distilled water was standardised by the bromate method[4]. A 0·2% solution of vanadophosphoric acid in distilled water and standardised by iodimetric method[5]. Beckman spectrophotometer Model DB was used for absorbance measurements.

Procedure for the Determination of Isoniazid

An aliquot of standard solution containing 10–375 μg of INH was transferred to a 25 ml volumetric flask. A 5 ml of 1M acetic acid and 6 ml of 0·2% vanadophosphoric acid were added and diluted to the mark with distilled water. The solutions were mixed well and absorbance was measured at 430 nm within 20 minutes against the corresponding reagent blank using a spectrophotometer. The amount of INH was then deduced from the previously prepared calibration graph.

Analysis of Isoniazid in Pharmaceutical Preparations

Twenty tablets were weighed and finely powdered. A weighed amount of the powder containing 5–20 mg of INH was dissolved in distilled water and filtered. The filtrate was made up to 100 ml and an aliquot of this solution was treated as described above for the determination of isoniazid.

For the syrup, 5 ml were diluted with water to get a final concentration of 200 μg/ml. The INH content in the diluted solution was determined as described above. The results of the assay of INH tablets and syrup presented in Table I compare favourably with the quoted values and those obtained by the official method of *U.S. Pharmacopoeia*[6]. The results of assay of tablets and syrup were cross-checked by N-bromo succinimide method[7].

TABLE I

Determination of isoniazid in commercial pharmaceutical preparations

Sample	INH content per tablet or ml (mg)			
	Label claim	Recovery		
		U.S.P. method	NBS method	Proposed method
Tablets				
Isotex forte[a]	300	296	293	295
Isonex[a]	100	101	99	100
Isokin[b]	100	97	95	96
Isokin-T forte[b]	300	294	294	295
Nydrazid[c]	300	294	292	294
Syrup				
Isokin[b]	20	19·6	20·2	19·2

* Average of five determinations.
[a] Marketed by Dumex.
[b] Marketed by Warner.
[c] Marketed by Sarabhai Chemicals.

Results and Discussion

INH forms 1 : 1 amber coloured complex with vanadophosphoric acid instantaneously in 0·1–1·2 M acetic acid medium at room temperature. Below 0·1 M acetic acid concentration, the maximum intensity is not obtained and above 1·2 M the species is less stable. The complex exhibits maximum absorption at 430 nm and stable for 20 minutes, afterwards the absorbance slowly decreases. The rate of formation and colour intensity of the product increased with increasing concentration of vanadophosphoric acid. The stability of the complex decreased on increasing the vanadophosphoric acid concentration which may be due to the oxidising effect of vanadophosphoric acid. The optimum amount of vanadophosphoric acid was 3–10 ml of 0·2% solution. Hence 6 ml of 0·2% vanadophosphoric acid and 5 ml of 1 M acetic acid in a total volume of 25 ml were used in all subsequent work. Beer's law is obeyed within the concentration range of 0.4–15 ppm. For $\log I_0/I = 0·001$, the sensitivity of the reaction as calculated

from the Beer's law data is 28·5 mg/cm² and the corresponding molar absorptivity of the complex is $4{\cdot}81 \times 10^3$ l mole^{-1} cm^{-1}.

The usual excipients like talc, starch, magnesium stearate, stearic acid, sorbic acid, glucose, lactose, gumacacia, gelatin and mannitol in amounts far in excess of their normal occurrence in pharmaceutical preparations did not interfere in the analysis of INH by the proposed method. Hence the method can be adopted for quick analytical control of INH tablets and syrup at the manufacturing stage.

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1. Stewart, J. T. and Settle, D. A., *J. Pharm. Sci.*, 1975, **64**, 1403.
2. Tan, H. S. I., *Ibid.*, 1973, **62**, 993.
3. Hashmi, M. H., Adil, A. S., Malik, F. R. and Ajmal, A. I., *Mikrochim. Acta*, 1969, **4**, 772.
4. Vulterin, J. and Zyka, J., *Chem. Listy*, 1954, **48**, 1745.
5. Heczko, T., *Z. Anal. Chem.*, 1926, **68**, 461.
6. *United States Pharmacopoeia*, **19**, p. 272.
7. Radecka, C. and Nigam, I. C., *Can. J. Pharm. Sci.*, 1966, **1**, 17.

SYNTHESIS OF SULPHENAMIDE, SULPHENIMIDE AND SULPHENIMINE WITH SULPHENYL BROMIDE OF ORTHO-MERCAPTO-AZO COMPOUND

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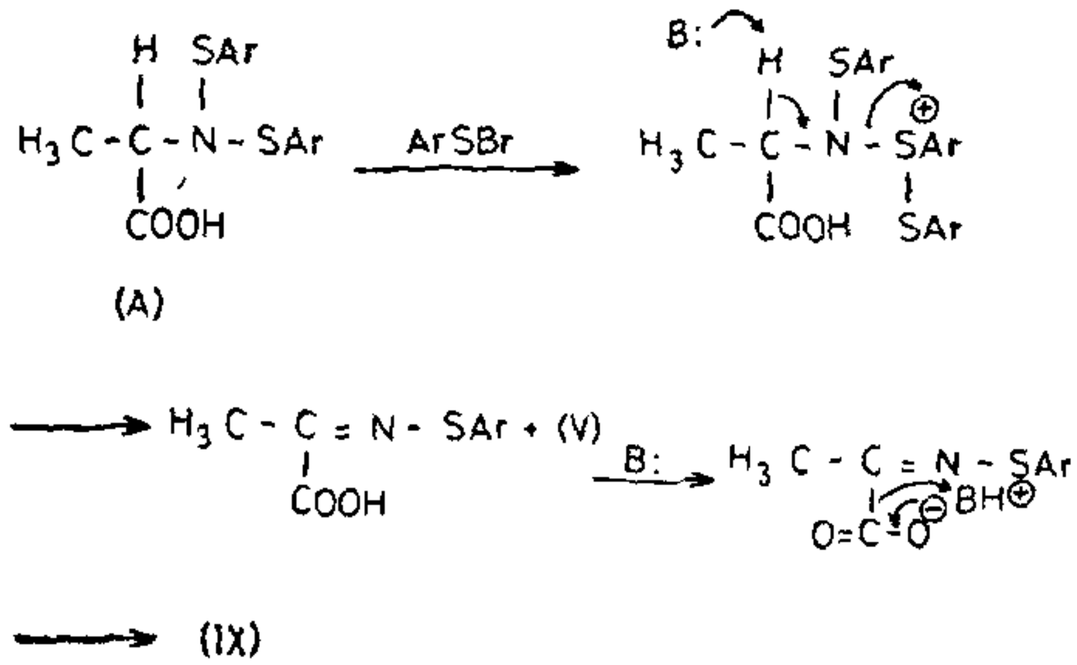
N-sulfhenylation of 2-naphthylamine, phthalimide, thiourea, and alanine with 4-dimethylaminoazobenzene-2′-sulphenyl bromida (I)[1] is studied. The rearranged product (II) dominates in the case of 2-naphthylamine, whereas the sulphenimine (IX) is the main product obtained from alanine. Thiourea undergoes S-sulphenylation (VII) and phthalimide gives the expected sulphenimide (VI).

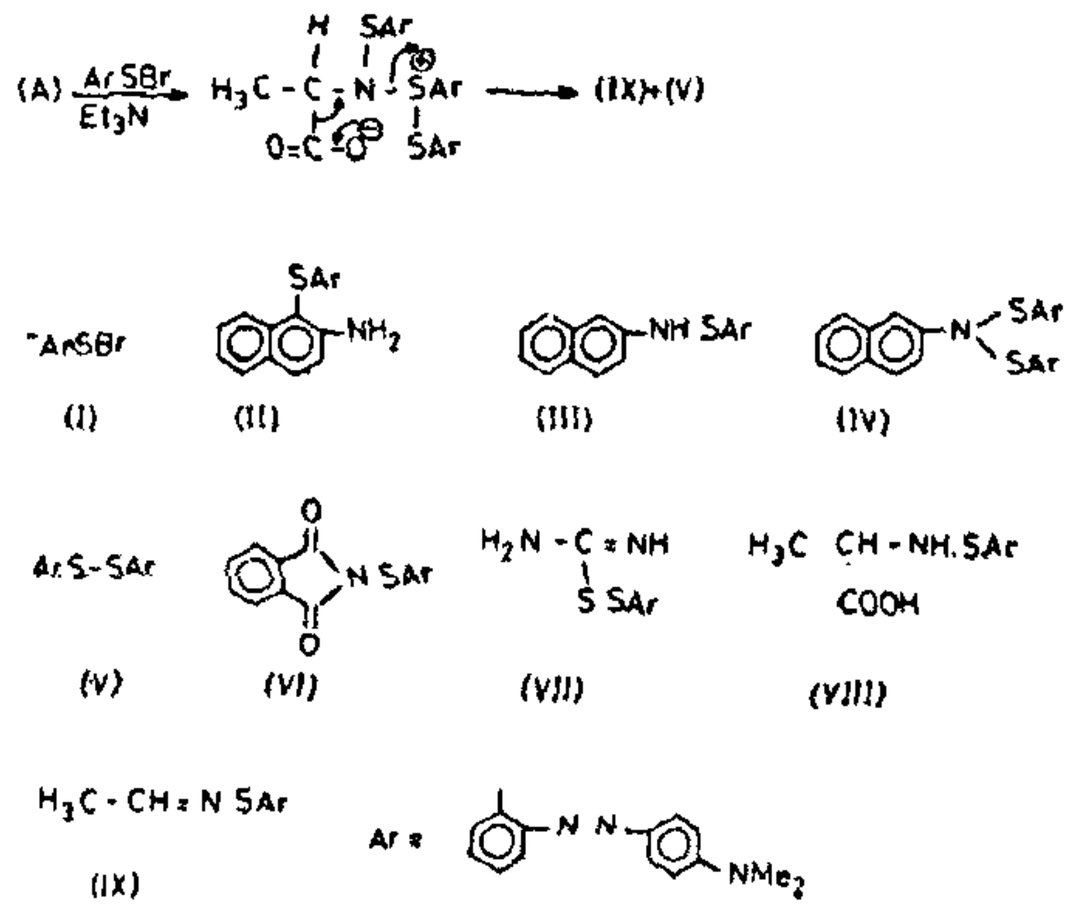
To investigate the utility of *o*-mercaptoazo compound in the preparations of sulphenamides, we have studied the reactions of 4-dimethylaminoazobenzene-2′-sulphenyl bromide[1] with 2-naphthylamine phthalimide, thiourea and alanine in a polar solvent in the presence of an acid scavenger triethylamine at room temperature. With 2-naphthylamine the diarylsulphide (II) is obtained as the major product. Small amounts of the sulphenamide (III), the di-N-sulphenylated product (IV) and the disulphide (V) are also obtained. N-sulphenylation of phthalimide is slow and forms the sulphenimide (VI) in a low yield, the disulphide being the other main product. Thiourea undergoes S-sulphenylation instead of N-sulphenylation giving a mixture of the disulphides (VII) and (V). From the complex reaction products of alanine the sulphenamide (VIII) is obtained only as a minor product. Its IR spectra show that N-sulphenylation of alanine does not destroy its zwitter ionic nature, though the characteristic amino acid band in the range 3000–2000 cm^{-1} disappears. The major product of the reaction is found to be the thiooxime (IX). The disulphide (V) is the third product identified.

The formation of (IX) may be rationalized as per Scheme 1 or 2.

Scheme 1



Scheme 2



Experimental

Reaction with 2-naphthylamine

To a solution of the sulphenyl bromide (I, 1·9 g) in ethanol (100 ml) was added a solution of 2-naphthylamine (400 mg) in ethanol (100 ml) and 4–5 drops