Discussion

The excretion of SU and SG in carbon tetrachloride treated rats seems to have been reduced presumably due to inhibition of their biosynthesis, by carbon tetrachloride induced inhibition of hepatic microsomal system which is responsible for conjugation of drugs with glycine and/or glucuronic acid. Phenobarbitone is known to enhance metabolism of salicylamide in human by hepatic microsomal enzyme induction[13]. It however failed to alter in any way the metabolic rate of sodium salicylate in normal rats. Yaffe *et al.*[13] have also reported similar observation. The difference in the effect of phenobarbital on the glueuronide conjugation of salicylamide and salicylate is of considerable interest. There have been other reports of similar differences. Remmer[14] found that phenobarbital increased the rate of glucuronide conjugation of sulfadimethoxine in rats but he did not obtain a similar barbiturate effect with salicylate and salicylamide[15]. Catz and Yaffe[16] found that sodium barbital enhanced the glucuronide conjugation of bilirubin in mice but had no effect on the conjugation of phenolphthalein and ortho-aminophenol. The reason for these selective effects is not known at present. They may be due to the existence of more than one glucuronide-forming enzyme system[17], each with its own substrate specificity and differing in their response to barbiturates.

Phenobarbitone, when given with carbon tetrachloride, failed to antagonise the inhibitory effect of carbon tetrachloride on salicylate metabolism. Conversely there was further decrease in urinary excretion of salicylate conjugates. Such potentiation of carbon tetrachloride effect is difficult to explain. It is however possible that this effect has relation with the depressant effect of phenobarbitone or its actions on metabolism.

Phenobarbitone is known to inhibit autonomous ganglion and also the cholinergic impulses. The studies on the effect of barbiturates on sympathetic neurotransmission are scant but are indicative of catecholamine release from synapses after barbiturate administration[18]. Such processes will have additive effect to carbon tetrachloride's hepatotoxic action which is believed to be predominantly due to catecholamine release. Miller[19] has also reported that barbiturates enormously sensitize to the toxicity of carbon tetrachloride.

Thus, the potentiation of carbon tetrachloride-hepatotoxicity by phenobarbitone resulting in reduced rates of salicylate metabolism and elimination would elevate salicylate blood levels and consequently lead to salicylate toxicity.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMIDINE SULPHIDES

Introduction

HIGH local anaesthetic activity and low toxicity have been claimed[1] for a group of pseudothiouronium salts. Sulphur substituents in these salts included 2-amino ethyl, 2-(1-piperidyl) and 2-(4-morpholinyl). Numerous 2-alkyl pseudothiourea hydrochlorides showed marked activity[2] against *Staphylococcus aureus* and

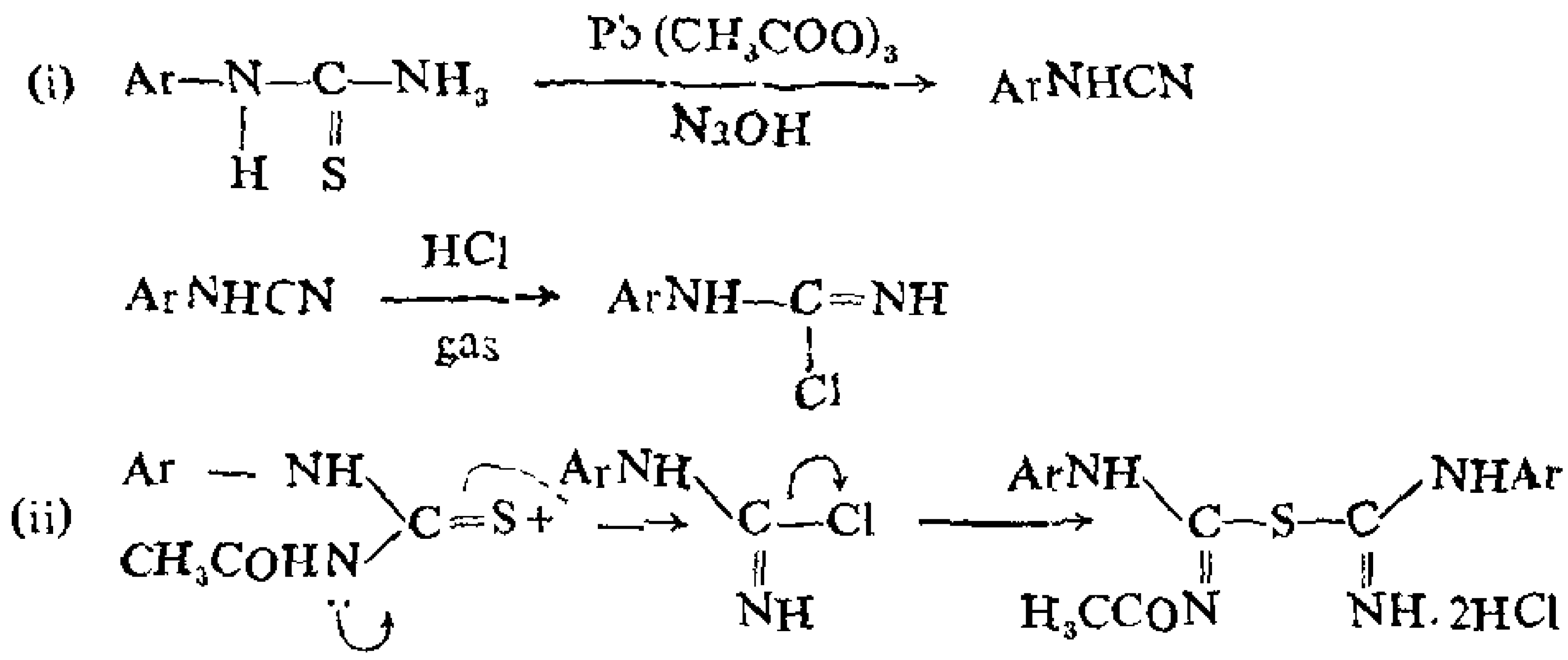
Eberthella typhi. Some of the 1, 3-di (susbstituted phenyl) thioureas had significant activity against several species of actinomyces and fungi[3]. Certain N-acetylthioureas are useful as sprays in protecting vegetable crops against many plant diseases caused by fungi and bacteria[4]. A number of aryl amidines have been found to be valuable for the treatment of protozoan diseases[5, 6]. So it was quite significant to synthesize certain amidine sulfides with acetyl moiety attached to the nitrogen of the thoiurea grouping and study their biological properties.

The aryl cyanamides were prepared by the method of Sahasrabudhey[7] by desulfurisation of aryl thioureas with alkaline lead plumbite solution. Acetylation of aryl thiocarbamides was done with acetic anhydride[8] and their m.pt. was confirmed with that reported in literature[9], Table I. The Aryl cyanamides were converted into respective amidine sulphides by passing dry hydrogen chloride into their chloroform solution. Acetylated aryl thioureas were dissolved in acetone and added gradually to the cold aryl amidine chlorides giving aryl amidine sulphide dihydrochlorides.

The aqueous solution of the hydrochlorides on addition of picric acid gave picrates. The physical poperties and analytical data are reported in Table II.

These compounds were formed by the nucleophilic displacement of the halogen of amidine by the sulphur nucleophile of the thiourea moiety (Scheme-1).

Scheme-1

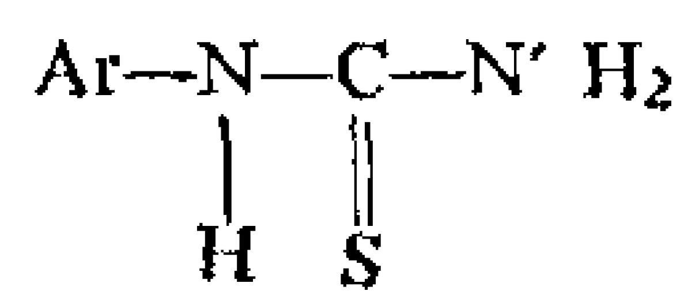


where Ar-phenyl, *p*-tolyl, *o*-tolyl, *p*-Cl-phenyl, etc.

The structure of the compounds was assinged on the basis of the elemental analysis, spectral data and an analogous reaction[10].

TABLE I

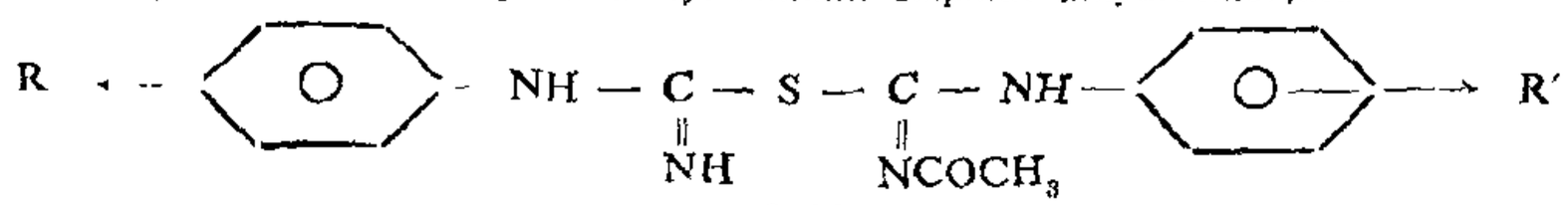
Melting points of some acetylated and non-acetylated aryl thiocarbamides



Sl. No.	Ar	Non acetylated aryl thioureas m.p. (° C)		N'-Acetyl aryl thioureas m.p. (° C)	
		Obs.	Reported	Obs.	Reported
1.	C_6H_5	154	154	176	176
2.	$4\ CH_3C_6H_4$	181	181	142	142
3.	$3\ CH_3C_6H_4$	112	112	180	..
4.	$2\ CH_3C_6H_4$	160	158	186	..
5.	$4\ ClC_6H_4$	169	139	172	186
6.	$4\ OCH_3C_6H_4$	183	185	150	..
7.	$4\ OC_2H_5C_6H_4$	163	165	195	144

TABLE II

Aryl formamidine N-Aryl-N'-acetyl amidine sulphide dihydrochlorides



Sl. No.	R	R'	M.P. (°C)	Yield (%)	Picrates M.P. (°C)	Molecular formula	Analysis		Equivalent weights	
								Cal.	Obs.	Obs.	Cal.
1.	H	H	149	51	128	$C_{16}H_{18}N_4OSCl_2$	C :	49·99	49·78	183·65	192·5
							H :	4 54	4·24		
							N :	14·54	14·71		
2.	H	$3CH_3$	129	58	127	$C_{17}H_{20}N_4OSCl_2$	C :	51·13	51·96	187·43	199·5
							H :	5·00	4·74		
							N :	14·00	14·24		
3.	H	$4CH_3$	135	62	87	$C_{17}H_{20}N_4OSCl_2$	S :	8·26	8·09	189·10	199·5
4.	H	$2CH_3$	134	50	62	$C_{17}H_{20}N_4OSCl_2$	C :	51·13	51·54	185·23	199·5
							H :	5·0	4·67		
5.	H	4Cl	153	65	131	$C_{16}H_{17}N_4OSCl_2$	C :	45·80	45·58	201·58	209·75
							H :	4·05	4·80		
							N :	13·40	13·66		
6.	H	$4OC_2H_5$	137	66	132	$C_{18}H_{22}N_4O_2SCl_2$	N :	13·04	13·42	203 89	214·50
7.	H	$4OCH_3$	145	68	137	$C_{17}H_{20}N_4O_2SCl_2$	S :	7·94	7·71	200·76	207·50
							Cl* :	17·10	17·68	..	..
8.	$4CH_3$	4Cl	132	67	130	$C_{17}H_{19}N_4OSCl_3$	N :	13 05	13·31	..	..
							S :	7·42	7·23	..	..
							Cl* :	16·49	17·02	..	..
9.	$4CH_3$	$4CH_3$	144	69	115	$C_{18}H_{22}N_4OSCl_2$	S :	7·75	7·53	..	..
10.	4Cl	$2CH_3$	155	58	125	$C_{17}H_{19}N_4OSCl_2$	S :	7·42	7·46	..	..
11.	$4OC_2H_5$	$4OC_2H_5$	132	64	139	$C_{20}H_{26}N_4O_3SCl_2$	S :	6·74	6·58	..	..

Cl* = Ionisable.

The infra-red of the compounds show a characteristic absorption for carbonyl group (>C=O) at 1680 cm^{-1}. Broad NH absorption falls in the region of 3400 cm^{-1} – 3100 cm^{-1}. Primary amide (–NH–C · CH_3) near

O

1620 cm^{-1}. The weak hydrogen bonding of $-N^+H$ causes the frequency to drop only slightly. The typical aromatic frequency vibration with their respective substituents patterns are evident in the region of 900 cm^{-1}–800 cm^{-1}. A weak frequency is noticed in the region of 680 cm^{-1} which may be possibly due to (>C–S–$C_{\lesssim}$) (Table III).

The compounds have been screened for their local anaesthetic activity on frogs at 0·1% concentration in water and compared with lidocaine hydrochloride of equal concentration. The amidine sulphide[2] formed by the condensation of phenyl amidine chloride and N-acetyl-N'–*m*'-tolyl thiourea was found to be most active The antifungal testing was carried on *Alternaria alternata* employing Czapek-Dox technique. All the compounds exhibited significant activity and inhibited the growth by 80–90%. These compounds were also tested for their antibacterial activity on *E.coli, Proteus vulgaris, Staphylococcus aureus* and *Streptococus faecalis* and were found inactive (Table III).

Experimental

All melting points were taken by capillary method and are uncorrected. The infra-red spectra were recorded on Perkin-Elmer 720 model by preparing KBr or Nujol mull. All aryl thioureas were prepared by mixing aromatic amine, conc. hydrochloric acid and ammonium thiocyanate isomerisation method[11], and m.pt. agreed with that reported in literature.

TABLE III

Biological properties and I.R. frequencies of Aryl formamidine-N-Aryl-N'-acetyl amidine sulphide dihydrochlorides

Compd. No. (as in Table II)	Local anaesthetic activity with 0·05 NHCl (in minutes)	0·1 NHCl (in minutes)	Antifungal activity	Antibacterial activity	I.R. frequencies
1.	9·0	9·0	+	—	1665 (>C=O), 1620 (>C=N– and amide II), 690 (>C—S—C<) 2300–3400 (—N⁺H), 1520, 1580 (Aromatic rings), 740 (monosubstituted)
2.	4·0	5·0	+	—	1600 (>C=O), 1620 (>C=N– and amide II), 690 (>C—S—C<) 2200–3600 (–NH⁺), 1500, 1590 (Aromatic rings), 740 monosubstituted), 840 (metadisubstituted).
3.	18·0	19·0	+	–	1680 (>C=O), 1620 (>C=N— and amide II), 685 (>C—S—C<), 1470, 1500 (Aromatic rings), 740 (monosubstituted), 850 (paradisubstituted), 2300–3400 (N⁺H).
4.	7·50	7·50	+	—	1678 (>C=O), 1620 (>C=N— and amide) 680 (>C—S—C<) 1500, 1560, 1600 (Aromatic rings), 740 (monosubstituted) 850 (paradisubstituted), 2300–3500 (N⁺H).
5.	6·0	9·0	+	—	1670 (>C=O), 1580 (>C=N— and amide II), 680 (>C—S—C<), 1500–1580 (aromatic rings), 740 (monosubstituted), 840 (paradisbustituted), 2300–3400 (—N⁺H).
6.	12·0	12·0	+	—	1679 (>C=O), 1620 (>C=N— and amide II), 680 (>C—S—C<), 1500, 1580 (aromatic rings), 740 (monosubstituted), 840 (paradisubstituted), 2300–3400 (—N⁺H).
7.	11·50	11·50	+	—	1680 (>C=O), 1623 (>C=N– and amide II), 681 (>C—S–C<), 1520, 1560 (aromatic rings), 745 (monosubstituted), 840 (paradisubstituted), 3100–3400 (—N⁺H).
8.	8·0	8·0	+	—	1720 (>C=O), 1620 (>C=N— and amide II), 740 (>C—S—C<), 1480, 1500 aromatic rings), 840 (paradisubstituted), 2300–3500 (—N⁺H).
9.	8·50	8·50	+	—	1685 (>C=O), 1620 (>C=N— and amide II), 710 (>C—S—C<), 810 (paradisubstituted), 1520, 1560, 1600 (aromatic rings), 2300–3500 (—N⁺H).
10.	9·0	13·0	+	—	1680 (>C=O), 1625 (>C=N— and amide II), 730 (>C—S—C<), 1500, 1580 (aromatic rings), 735 (orthodisubstituted), 840 (paradisubstituted), 2300–3500 (—N⁺H).
11.	10·0	10·0	+	—	1690 (>C=O), 1630 (>C=N— and amide II), 730 (>C—S–C<), 1500, 1580 (aromatic rings), 835, 880 (*p*-disubstituted) 2300–3500 (—N⁺H).

Chemical

N-Aryl-N'-acetyl thiourea (Table I)—Phenyl thiourea (5·0 g) was dissolved in minimum quantity of glacial acetic acid and acetic anhydride (5·0 ml) was added. The resulting mixture was refluxed for half an hour, cooled and poured over crushed ice-water. The solid filtered, crystallized from ethanol, m.pt. 176 C. Similarly other aryl thioureas were prepared (Table I).

Aryl amidine chloride—Phenyl thiourea (5·0 g) was dissolved in hot water and a mixture of NaOH (5·0 g) and lead acetate (5·0 g) was added to the hot solution of phenyl thiourea. The whole content was heated on a water bath for one hour. The lead sulfide was filtered and the filtrate was acidified with cold acetic acid. The phenyl cyanamide was extracted with chloroform and dried over anhydrous sodium

sulphate. Dry hydrogen chloride was passed into the chloroform solution giving the phenyl amidine chloride. Similarly other amidine chlorides were prepared.

Amidine sulphides—To a solution of this amidine chloride in chloroform was added a solution of N-acetyl-N'-phenyl thiourea (2·0 g) in acetone. For completion of the reaction further hydrogen chloride was passed in the reaction mixture. After some time a crystalline product seperated, m.pt. 149 C. The product could not be recrystallized without decomposition and desulphurised alkaline lead acetate solution. The aqueous soluiton of the compound gave a picrate with picric acid, m.pt. 128 C. Other amidine sulphides were also prepared similarly and their physical properties are given in Table II.

Biological

(i) *Local anaesthetic activity*—Frogs were used for local anaesthetic activity by nerve block method. A transverse incision is made on the abdominal wall and Viscera (through this opening) is removed carefully to expose the lumber plexus. Frog is hanged by its lower jaw. The solution of the compound (0·1 g/100 ml) is poured into this pocket and the foot withdrawal response with 0·1 NHCl and 0·05 NHCl is tested. The time required, when the foot withdrawal is completely stopped, is noted (Table III).

(ii) *Antifungal testing*—The fungal testing was done on *Alternaria alternata* employing Czapek-Dox liquid growth medium mixed with the amidine sulphides (0·1% solution). The growth was noticed after twelve hours by hanging drop method for spore germination. The percent inhibition of germinating fungi was determined (Table III).

(iii) *Antibacterial testing*.—Different bacterial cultures were taken and sub-cultured on nutrient agar plates. Filter paper discs (3–4 mm diameter) soaked in the amidine sulphiade solution (200 wg) were fixed at different places. Next day after 24 hrs of incubation sensitivity was observed. All the compounds were found to be inactive.

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EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF ATROPINE SULFATE BY SOLOCHROME GREEN V 150

THE analysis of atropine is of particular interest to pharmaceutical and forensic chemists and toxicologists. Many of the commonly employed methods are indirect and time-consuming[1–4]; the precipitation methods are only applicable with large amounts of atropine. The range of absorbance by the picrate method is small. The iodine method gives a very stable colour but does not permit the use of standard curves. A few direct spectrophotometric methods are available that make use of acidic dyes which form extractable ion pair complexes with the basic alkaloids. Generally, triphenylmethane dyes are used for the determination of atropine[5–7]; very few azo dyes[8] have been employed for its determination. The author, in his investigations on the use of diazo dyes for the determination of alkaloids, found that these dyes are more sensitive than the commonly employed triphenylmethane dyes and proposed methods for strychnine[9] and brucine[10]. The present communication describes the microdetermination of atropine sulfate by a diazo dye, Solochrome Green V 150 (I) supplied by ICI, Calcutta. The method is rapid, sensitive and applicable to 2 μg/ml of the solution.