2, 3, 5-TRIPHENYLTETRAZOLIUM CHLORIDE AS A NEW REAGENT FOR THE DETECTION OF SULPHIDE AND SOME ORGANIC THIOCOMPOUNDS

U. MURALIKRISHNA, K. V. BAPANAIAH AND G. V. RAMANADHAM
Department of Chemistry, Andhra University, Waltair, India

INTRODUCTION

FEIGL[1,2] described the detection of submicrogram quantities of organic sulphur compounds based on their catalytic action on the reaction between iodine and azide. The detection of sulphide was affected by using sodium nitroprusside[3], dimethylaniline, *p*-phenylene diamine[4] as chromogenic reagents. Several chromatographic[5,6] and crystallographic[7] methods also had been reported. A survey of the literature shows very few[8,9] reports for the detection of sulphur in organic compounds. Recently, Feigl critically reviewed the reagents for the detection of other sulphur compounds[10]. Sodium plumbite was used for the detection of thioacetamide. Phosphomolybdic acid, *p*-nitrosodimethyl aniline, pentacyanoammine ferroate and 2, 6-dichloroquinone-4-chloramine are the reagents used for thiosemicarbazide. Raney alloy, sodium nitroprusside, phosphomolybdic acid, selenous acid are used for dithioxamide. Most of the methods described using the reagents mentioned, for the detection of sulphur compounds are time consuming and involve heating on steam bath. These methods are susceptible to the interference of thiourea, thioglycollic acid, semicarbazide, urea and hydroxylamine. In this communication we report the use of 2, 3, 5-triphenyltetrazolium chloride for the detection of the sulphide, thiosemicarbazide, dithiooxamide and thioacetamide in weakly alkaline medium. The new method involves the extraction technique and has the advantage that it can be carried out in the presence of urea, thiourea, semicarbazide, hydroxylamine thioglycollic acid and anions like chloride, bromide, iodide, acetate, oxalate and citrate.

EXPERIMENTAL

Reagents.—0·1% solution of 2, 3, 5-triphenyl tetrazolium chloride was prepared by dissolving G.R., E. merck grade sample in deionized water. The solution must be stored in a dark place as its exposure[11,12] even to diffused light on long standing will result in the formation of the coloured product.

0·1% solution of sodium sulphide, thioacetamide, thiosemicarbazide and dithiooxamide were prepared by dissolving Analar, B.D.H. samples in deionized water

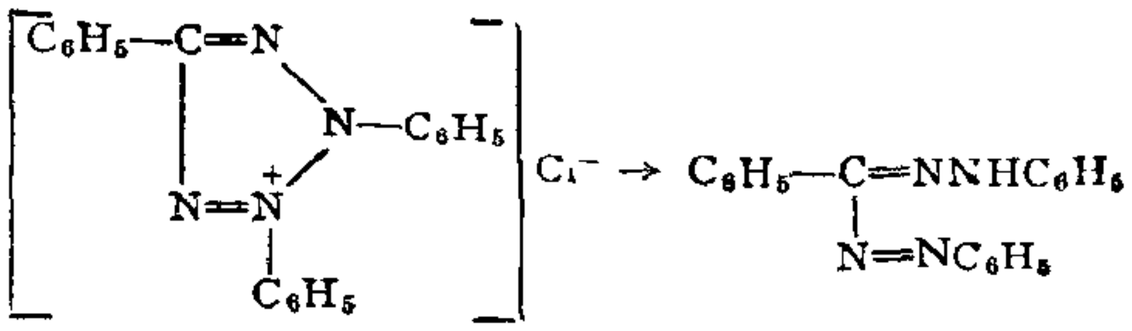
B.D.H. Analar quality methyl isobutyl ketone was used without further purification.

PROCEDURE

0·1 ml test solution is mixed with 0·2–0·4 ml of sodium hydroxide (0·02 M) and 0·2 ml of 2, 3, 5-triphenyl tetrazolium chloride (0·1%) in a semimicro test tube, and the volume was made to 1 ml with deionized water. The mixture is equilibrated with 0·2 ml of methyl isobutylketone for 1–2 minutes. The appearance of the reddish brown colour in the extract after equilibration shows the presence of the sulphur compounds.

RESULTS AND DISCUSSION

Table I gives the identification and dilution limits of various sulphur compounds. Under the experimental conditions the reagents do not give any colour in the organic phase even after 10 hours at 35° C in the absence of sulphur compounds. In alkaline medium, the sulphur compounds other than sulphide may hydrolyse resulting in the formation of sulphide, which in turn reacts with the reagent resulting in the reddish brown coloration in the extract. The extract was found to be triphenyl formazan[13,14] as its spectrum exhibits maximum absorption band in the region of 480–490 nm.



2, 3, 5-Triphenyl Tetrazolium Chloride — Triphenyl Formazan

TABLE I

Identification and dilution limits

Sulphur compound	Identification limit μg in 1 ml	Dilution limit
Sodium sulphide ..	1·0	$1 : 1\times10^5$
Thioacetamide ..	10·0	$1 : 1\times10^4$
Thiosemicarbazide ..	2·0	$1 : 5\times10^4$
Dithio oxamide ..	5·0	$1 : 2\times10^4$

INTERFERENCES

The amounts of ions and substances indicated did not effect the test.

5000 ppm of Cl^-, Br^-, I^-, oxalate, citrate, sulphite, phosphate, nitrate, nitrite, tartrate, carbonate azide, acetate, bromate and 1000 ppm of urea, hydroxylamine, semicarbazide, thiourea, glucose, starch, 50 ppm of thioglycollic acid do not interfere, while even minute quantities of thiocyanate, perchlorate, periodate and iodate obscure the colour formation.

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OCCURRENCE OF AFLATOXINS AND CITRININ IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) AT HARVEST IN RELATION TO POD CONDITION AND KERNEL MOISTURE CONTENT

P. SUBRAHMANYAM AND A. S. RAO*

Microbiology Laboratory, Department of Botany, S.V. University, Tirupati 517502 (A.P.), India

ABSTRACT

Groundnut pods were collected from fields on the day of harvest in November, 1972, graded into undamaged and damaged pods and kernel moisture content was determined. The accumulation of a yellow pigment in some groundnut kernels, especially in damaged pods, was noticed and it was identified as citrinin. Only *Aspergillus flavus* isolates were found to produce aflatoxins while isolates of *Penicillium citrinum*, *P. jenseni* and *A. terreus* produced citrinin. High levels of aflatoxins and citrinin were associated with kernels having less than 30% of moisture, which occurred under rain-fed conditions. Where the moisture content of kernels is high (under irrigation) there was very little or no formation of the mycotoxins. In all the cases damaged kernels were found to contain the toxins. Kernel moisture content and pod damage appear to be the major governing factors for fungal infestation and toxin accumulation before harvest.

INTRODUCTION

PRESENCE of aflatoxins in groundnut kernels before harvest[8] and at the time of harvest[6 14] had been reported. Their presence was attributed to low kernel moisture content (30%), over maturity, unfavourable weather conditions and excessive pod damage. *Aspergillus flavus* Link. invades groundnut pods in the field before harvest, during storage or during handling[5·6·13 15]. Optimum kernel moisture content of 20–30%[2] and temperature of 25°–35° C[9] are favourable for aflatoxin production. Kernels are more susceptible to fungal infestation and toxin production when their moisture content is between 10 and 30%[8].

During our studies on fungal infection of groundnut and aflatoxin accumulation before harvest, we found an accumulation of a yellow pigment in groundnut kernels, especially in kernels from rotted pods (Fig. 1). Such kernels emit golden yellow fluorescence when exposed to UV light (Fig. 2). The chemical nature of this substance was determined and the conditions for its accumulation was also investigated.

MATERIALS AND METHODS

Groundnut samples were collected from the fields around Tirupati (A.P.), on the day of harvest in November, 1972 from three different localities. Two samples of 1 kg of pods for each were collected in polythene bags. The pods were graded into undamaged (sound mature) and damaged pods

* Present address: Professor of Botany, Andhra University, P.G. Centre, Guntur-5 (A.P.), India.