and enables one to overcome the apparent shortcomings attributed to the *in vitro* method of assay. The additional advantage that the cells need no other supplement of the coenzymes for routine assay of the enzyme activity *in situ*, makes this procedure uniquely distinct even over the other *in situ* assay methods known, where the only advantage over the conventional *in vitro* methods is that whole cells can be used in place of cell-free preparations. All our subsequent studies on the regulatory phenomena controlling the biosynthesis of NAR in *C. utilis*, which involve handling of innumerable concentrations, and assay of NAR activities at brief time periods, are conducted using the *in situ* technique and the results appear elsewhere.

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A RAPID SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF DIOSGENIN IN *DIOSCOREA* TUBERS

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ABSTRACT

A rapid method for the assay of diosgenin in *Dioscorea* tubers has been developed Simultaneous hydrolysis and extraction of the tubers (50 mg) by 3 N HCl and hexane is carried out in 1½ to 2 hours. $SbCl_5$ (24% in $HClO_4$) has been used for developing colour. The red colour formed is stable for 2 hours and is evaluated spectrophotometrically at 486 nm. One analyst can assay 8 to 10 samples per day by this method.

INTRODUCTION

DIOSGENIN is used in the synthesis of steroidal drugs. Several analytical procedures are available for the estimation of diosgenin in pure state or in *Dioscorea* tubers. Gravimetric procedure[1], gas liquid chromatography[2], colorimetry[3], densitometric thin-layer chromatography[4] and infra-red spectrometry[5] have been used. These methods involve more time, labour and material. The present paper describes a rapid method combining hydrolysis and extraction of *Dioscorea* tubers with spectrophotometric method for the determination of diosgenin in the extracts. The distinguishing features of this method are (*a*) small quantities (50 mg) of the dried plant material required, (*b*) hydrolysis and extraction achieved in 1½ to 2 hours, (*c*) avoiding additional solvents[3-6] and (*d*) use of smaller quantities of antimony ion for the colour reaction.

EXPERIMENTAL

Pure diosgenin was isolated from *Dioscorea deltoidea* and purified by preparative T.L.C. using $SbCl_5$ solution. (24% in 70% $HClO_4$) for detection.

Procedure

(*i*) *Preparation of Standard Curve*.—25 mg of pure diosgenin were dissolved in 250 ml hexane (Solution A) and 0·75 ml and 1·0 ml of this solution were taken in two tubes and the solvent removed by bubbling air. $HClO_4$ (5 ml) followed by 0·1 ml of $SbCl_5$ solution. (24% in 70% $HClO_4$)

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were added to each tube. The mixtures were kept for half an hour. Absorbance was noted for both the solutions from 400 to 600 nm against reagent blank. A maximum absorption was noticed at 486 nm.

Volumes corresponding to 10, 20, 30, 40, 50, 80 and 100 µg of diosgenin were taken from solution A into different tubes, solvent removed and the colour developed as mentioned above. The absorbance of each was noted at 486 nm against reagent blank.

(*ii*) *Hydrolysis and Extraction*.—Finely powdered tuber was dried at 105° C and passed through 72 mesh sieve. This material (50 mg) and 25 ml each of 3 N HCl and hexane were refluxed in an all-glass apparatus on a magnetic stirrer hot plate for 2 hours. The temperature of the liquid was maintained between 90–96° C. The mixture was allowed to cool and the aqueous phase was given two shakings with 25 ml of hexane each time and combined organic phase was washed with 1% $NaHCO_3$ solution and subsequently with distilled water and the volume made upto 100 ml with hexane. From suitable aliquots of this extract (usually 1 ml) solvent was removed and the colour developed as described above and the absorbance measured at 486 nm against the reagent blank. Gravimetric procedure[1] was also undertaken to find out the accuracy and efficiency of this method.

Results and Discussion

Pure diosgenin when treated with $SbCl_5$ (24% in 70% $HClO_4$) showed λ_{max} at 486 nm. The red colour had a maximum absorbance after half an hour (23°–27°) and was stable for nearly two hours.

A straight line was obtained when the concentration of pure diosgenin (10, 20, 30, 40, 50 and 80 µg) was plotted against absorbance (Fig. 1). The colour reaction obeys Beer's Law at room temperature.

In the colorimetric procedure[3] for the estimation of pure diosgenin by a solution of $SbCl_3$ in a mixture of nitrobenzene and methanol, the solution is to be heated for at least 20 minutes at 60° C to develop the colour. Sofowora and Hardman[6] have developed a spectrophotometric method for the estimation of diosgenin in crude plant extracts. The procedure for obtaining crude plant extract has also been described[7]. In this method it takes more than 40 hours to obtain the crude plant extract which includes hydrolysis of the tuber, elimination of the acid, drying and extraction. Moreover, the stability of the red colour varies with time[6]. Reagents and tuber also required in larger quantities.

In the present method, only 50 mg of the dried material are adequate for the estimation. Both hydrolysis and extraction can be completed in about 2 hours. The quantitative aspect of the extraction has been confirmed by TLC of the aqueous layer. Hydrolysis conditions were thoroughly checked to make certain that the release of diosgenin was complete. In order to speed up the assay, advantage was taken of the immiscible character of hexane with aqueous phase. Hexane was found to be the most suitable of all the solvents tried. Only 0·1 ml $SbCl_5$ (24% sotution in 70% $HClO_4$) was found to give more stable colour as compared with 5 ml $SbCl_3$ (25% in $HClO_4$) used elsewhere[6].

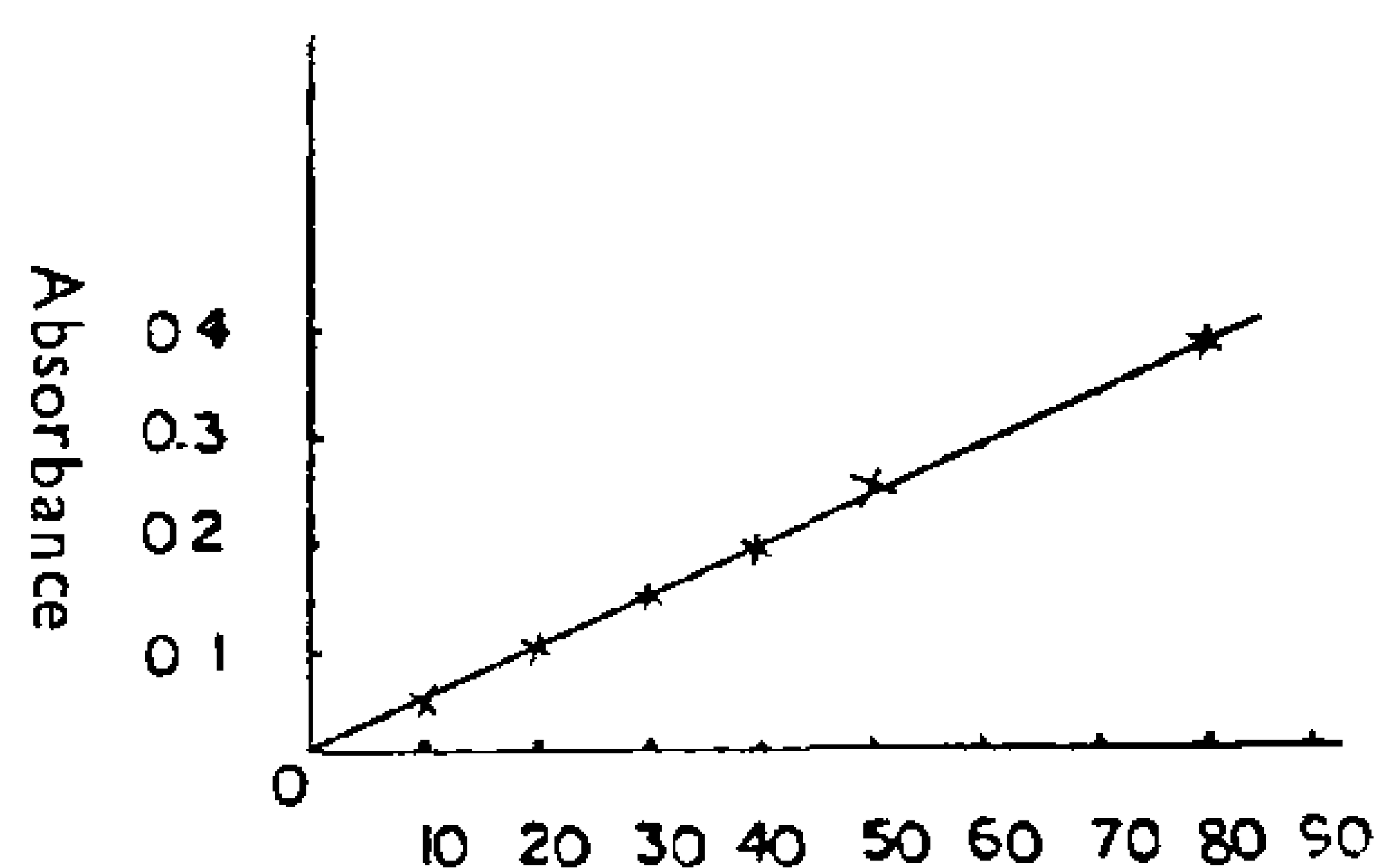

Fig. 1. Diosgenin concentration (µg).

The analytical results obained by gravimetry[1] and by the present method were nearly the same within the limits of experimental error (standard error = 0·15). The Students' *t* Test confirmed these observations. By the present method the analysis of 8 to 10 samples can be completed by one analyst in a day.

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