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THE IDENTIFICATION AND ESTIMATION OF THE MUSTARD-OIL GLYCOSIDES (SINALBIN AND SINIGRIN)

T. K. Obidairo

Department of Biochemistry, University of Benin, P.M.B. 1154, Benin City, Nigeria

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A technique is described for the chromatographic separation of sinalbin and sinigrin on paper and thin layer plates. A colourimetric method for the quantitative estimation of mustard oil glycosides has also been developed.

Key words: Mustard oil, Glycosides, Chromatography, Ninhydrin.

INTRODUCTION

There are no published chemical methods for the detection and estimation of the mustard-oil glycosides. The method employed by Hughes [1] of fermenting the glycoside and smelling the oil of mustard is unsatisfactory and unreliable. The author has developed entirely new methods for the qualitative detection, chromatographic indentification and colorimetric estimation of these glycosides. Black mustard (*Sinapis nigra*) seeds contain sinigrin and the enzyme myrosin while white mustard (*Sinapis alba*) contains the same enzyme and the glycoside sinalbin. Mustard preparations have been used to counter the irritation in neuralgia, rheumatism and pleurisy. Black and white mustards are mixed in the preparation of bath mustards, mustard bran, (a counter irritant) and mustard flour. Mustard flour, as a condiment, reflexly increases the flow of saliva and gastric juice and an appropriate dose is used as an emetic.

It is known that when compounds containng primary or secondary amino groups attached to an aliphatic carbon atom are heated at 100 – 110° with ninhydrin, colours (usually purple) are produced. When mustard-oil glycosides are treated with HCl they give isothiocyanate which gives mustard its characteristic odour. Heated further with acid, the isothiocyanate gives an amine.

It was thought that if a mustard-oil glycoside was heated sufficiently long with HCl, the formation of the amine and the estimation with ninhydrin could be effected in one stage provided that the medium was made neutral before the introduction of the ninhydrin since this only produces a stable colour under these conditions. In the present communication results of a method developed for the estimation of sinablin and sinigrin are described.

MATERIALS AND METHODS

Colour reagents

Antimony trichloride reagent: 20% w/v antimony trichloride in chloroform (2, 3).

Anisaldehyde reagent: 0.5 ml *p*-anisaldehyde was added to 50 ml glacial acetic acid and to this mixture was added 1 ml of conc. H_2SO_4.

Ninhydrin. 5 % in ethanol.

Phosphate buffer. disodium hydrogen phosphate/ potassium dihydrogen phosphate M/15; pH 7.0.

Chromatographic solvents

Solvent (A) *n*-butanol/methanol/water (25/25/10 v/v). This was used with Whatman No. 1 chromatography paper.

Solvent (B) *n*-butanol/methanol/ethyl acetate (20/40/ 40 v/v).

Stock standard glycoside solution' A solution of 500 μg/ml was prepared in 0.1 N HCl.

METHODS

(a) *Paper chromatography of the glycosides.* This was carried out with system A on Whatman No. 1 paper by the ascending technique. The total length of the run was 18 cm and the chromatograms were examined under U.V. light and then sprayed with the antimony trichloride reagent. It was heated at 70 – 80° for 5 min. The chromatogram was again examined under UV light.

(b) *Thin layer chromatography.* This was carried out on silica gel plates using solvent system B. The plates were

sprayed with the anisaldehyde reagent and heated at 100 - 100° for 5 min.

(c) *Estimation.* Equivalents of 50, 100 and 200 μg 500 μg of the glycoside were introduced into graduated test tubes and the volumes of the solutions made up to 1 ml with 0.1 N HCl; 1 ml of the acid being used as a blank. The tubes, covered with bubble condensers were placed in a boiling water bath for 1 hour. They were then removed and 4 ml of phosphate buffer added and the contents mixed. 0.5 ml of ninhydrin reagent was added, the tubes shaken, covered with bubble condensers and heated in a boiling water bath for 1 hour. To the cooled tubes was added 5 ml of *n*-butanol which on shaking removed the purple colour. The UV absorption spectrum of the 100 μg sample is shown in Fig. 1. The optical density (O.D) at 570 nm of all the samples was read, the results being shown in Fig. 2. The procedure was applied to sinalbin and sinigrin. The results obtained with the former being shown in Fig. 3.

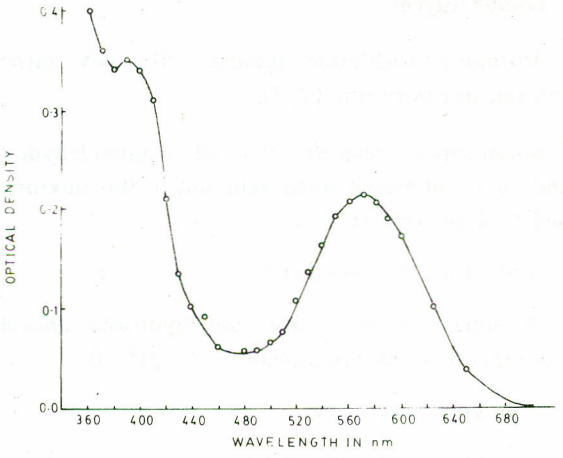

Fig. 1. Absorption spectrum of colour produced by Sinigrin when treated with ninhydrin reagent.

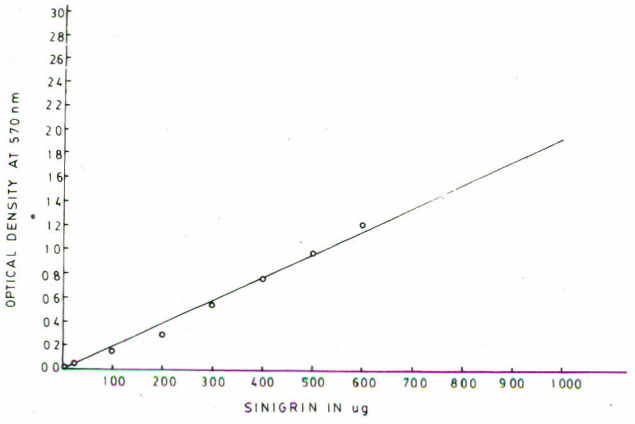

Fig. 2. Relationship between the optical density at 570 nm and the concentration of Sinigrin.

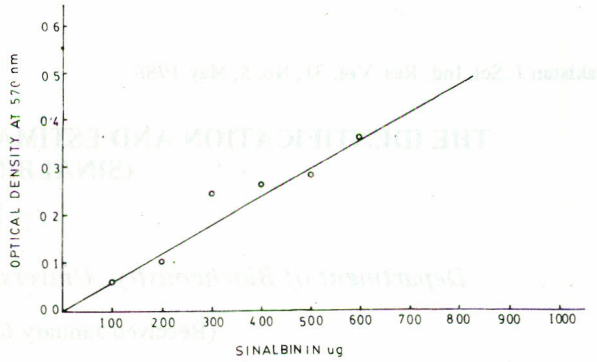

Fig. 3. Relationship between optical density at 570 nm and the concentration of Sinalbin.

(d) Application of the method to sinalbin extracted from *Sinapis alba.* 3 gm of the crushed seeds were extracted (3 times) with light petroleum ether (60 – 80°) and ether respectively; the extracts were discarded. The residue was added to boiling water and heated gently for 10 min. The cooled extract was filtered, evaporated to small volume under reduced pressure, chromatographed in solvent system A and the eluted sinalbin estimated by the above methods.

RESULTS

(a) *Paper chromatography.* Sinablin gave a fluorescent spot R_f 0.63 and a yellow spot with the same R_f after spraying with antimony trichloride. The spot showed a blue fluorescence under U.V. light after spraying. Sinigrin gave a yellow spot R_f 0.4 with antimony trichloride, this spot absorbed under the U.V. light.

(b) *Thin layer chromatography.* On treatment with the anisaldehyde reagent, sinablin gave a yellow green spot and sinigrin a yellow spot. Both have different R_f values.

(c) *Estimation.* There was a linear relationship between the concentration of the glycoside and the O.D. at 570 nm. The concentration of sinalbin in *Sinapis alba* was found to be 4%.

DISCUSSION

Up to date there are no published chemical methods for the detection and estimation of mustard-oil glycosides. In this work, solvent systems and locating agents have been developed for the paper and thin layer chromatography of sinigrin and sinalbin. The two glycosides give different R_f values and different colours when treated with the antimony trichloride and anisaldehyde reagents.

A method for their estimation which is specific when used in conjunction with chromatography has been deve-

loped. This is an entirely new method of estimation of three glycosides and is superior to the fermenting method employed by Hughes [1]. When used in conjunction with chromatography, contamination with amino acid can be avoided. The method has been successfully applied to sinalbin extracted from *Sinapis alba*.

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