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Isolation of Glucotropaeolin from Salvadora *Persica* L

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Summary: Glucotropaeolin was isolated and identified from the roots of Salvadora *Persica* L. Benzyl isothiocyanate was separated by the enzymatic hydrolysis of the glucosinolate. Benzyl nitrile was also detected in the hydrolysate. The presence of the latter compound further substantiates the possible formation of the isothiocyanate, thiocyanate or/and the nitrile derivatives from the thiazarine intermediate.

Salvadora *persica* L. is sporadically used in many countries in curing different diseases[1]. It has a wide spread usage for human medicine and personal hygiene, and the demonstration that a long history of use in folk medicine has a basis in pharmacological activity[2]. The roots of the plant is used by Moslems as a substitute of the tooth brush[3,4]. Ahmed and co-workers[5] reported the sporadic appearance of glucosinolates in some species belonging to certain families including Salvadoraceae. Ezmirly *et al*[2] succeeded in isolating some compounds from the plant roots in addition to S_8 (γ-monoclinic form 4.73%). The high content of elemental sulfur as well as the pungent odour of the plant stimulated us to search for the possible presence of other organic sulfur compounds in the plant.

Identification of glucotropaeolin (benzyl glucosinolate) as the only glucosinolate from the fresh and dried roots was confirmed through the preparation of the tetraacetate derivative, as well as by enzymatic hydrolysis.

The purified glucosinolate upon enzymatic hydrolysis by myrosinase at pH 5.6 yielded a steam-distillable oil. Subjecting the latter oil to GLC technique revealed that it is composed of benzyl nitrile (10%) and benzyl

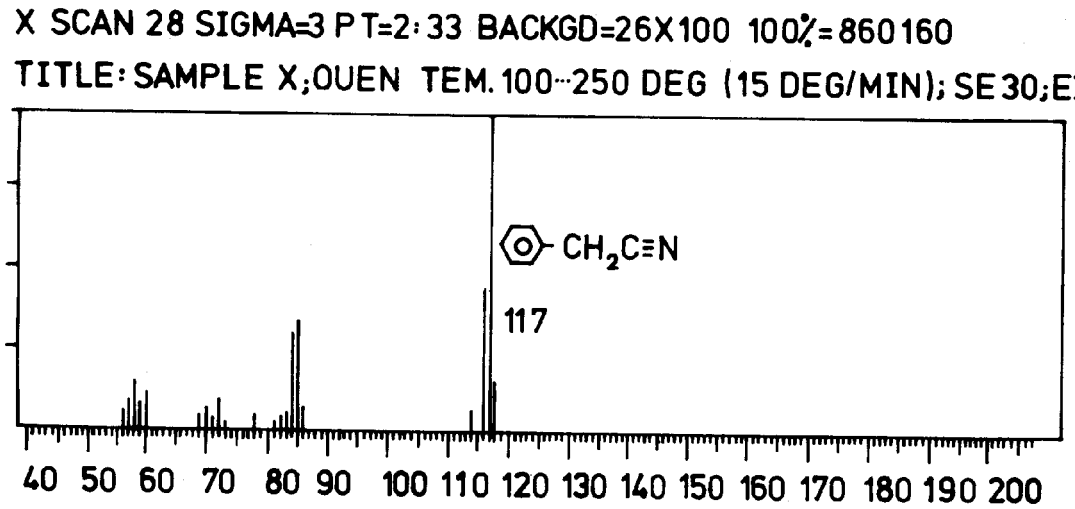

Fig.1

isothiocyanate (ITC) 90%.

GC-MS for the purified oil revealed two peaks at scan 28 and 80 which correspond to *m/e* 117 (benzyl nitrile) and *m/e* 149; as expected; for benzyl ITC respectively.

MS for the first compound (scan 28) revealed a peak at *m/e* 91 (pH. CH_2^+) and a base peak at *m/e 117 (pH.* CH_2CN) (Fig. 1). Furthermore, the MS for the second compound (scan 80) revealed a peak at *m/e* 72; a peak characteristic for all isothiocyanates[6], as well as the base peak at *m/e* 149 corresponding to the benzyl *iso*thiocyanate (Fig. 2).

The IR of the compound detected at scan 28 indicated the presence of CN group (an intense peak at 2200 cm^{-1})[7]. The NMR spectrum in $CDCl_3$ showed a downfield signal at 3.76 corresponding to the $-CH_2-$ protons of benzyl nitrile.

In addition to the expected SO_4^{-2} and glucose, elemental sulfur was detected in the hydrolysate mixture. Therefore, the presence of benzyl nitrile is further substantiated by the detection of elementary sulfur in the hydrolysate.

It is known that a glucosinolate releases its isothiocyanate, thiocyanate or nitrile constituents depending on the pH of the media[8,9]. More recently however, Carmark[10] postulated that these products could arise from a common thiazirene intermediate (Fig. 3).

The finding that benzyl isothiocyanate and benzyl nitrile were both detected in the hydrolysate at pH 5.6 is of special interest since the previous reports indicate that the isothiocyanate is the only compound released at this pH. Moreover, the isolation of glucotropaeolin from s. *persica* is recorded for the first time.

The influencial role of pH on the hydrolytic processes of glucosinolates is under investigation.

Experimental

M.p.s. were taken with a Kofler hot-stage microscope. IR spectra were recorded with a Pye Unicam SP 1025 spectrophotometer in Nujel and bands noted are either strong or medium in intensity. NMR spectra were recorded with Varian T 60 spectrophotometer. GC-MS were recorded with Riber May R 100 Hz.

Plant Material: Salvadora *persica* L. was collected from Wadi Abo Al-Rack, north of Taif, Saudi Arabia and was kindly authenticated by Prof. A.M. Migahid, Botany Department, College of Science, University of Riyadh.

Preparation of the glucosinolates:

a) From dry roots[11]: 1 Kg of the ground roots

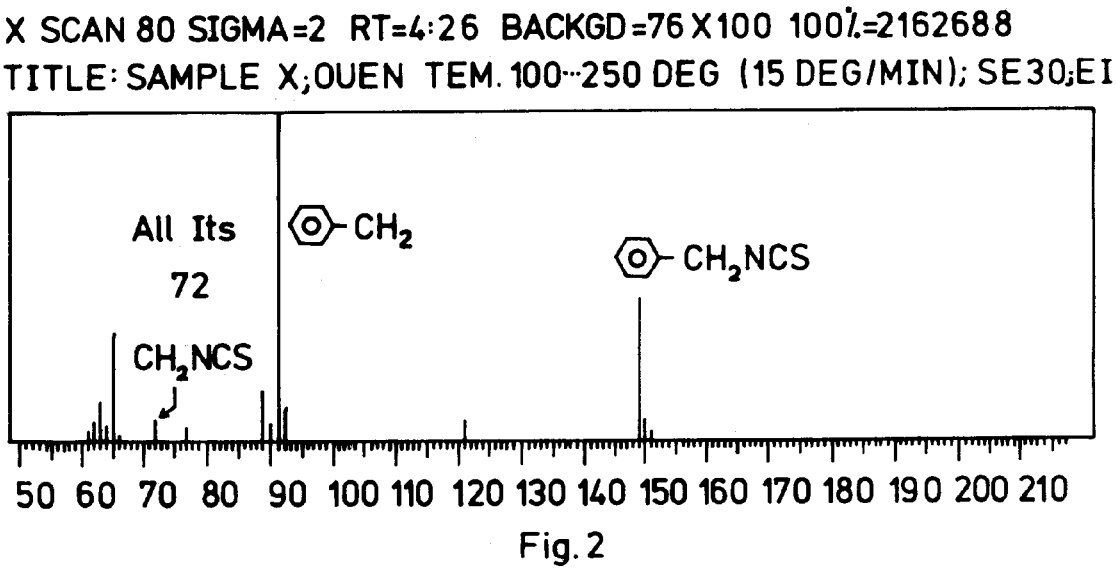

Fig. 2

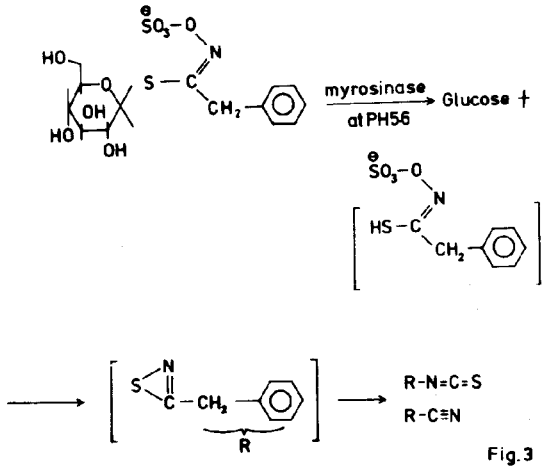

Fig.3

was extracted in a continuous extraction apparatus with 90% MeOH. The extract was dried at 50°C under reduced pressure. The dry residue was dissolved in MeOH (1 l) and chloroform (2.6 l) was added till complete precipitation was attained. The precipitate; containing glucosinolate (s); was re-dissolved in MeOH (1 l) and chloroform was added till complete precipitation. These steps were repeated twice again and traces of the glucosinolate (s) were detected in the filtrate. The final precipate amounted to 9 gm.

b) From fresh roots[12]: 1 Kg of the fresh roots was chopped and extracted with boiling MeOH (70%) and $Ca(CO_3)_2$ was added to stop the enzymatic action. The concentrated extract (1 l) after cooling and removing of the deposited substance, was diluted with MeOH to 2.5 l., boiled with activated charcoal for 20 min. The pale yellow filtrate was evaporated *in vacuo* to dryness (6 gm).

Chromatographic investigation:Descending PC (Whatman No. 1) was tried using different solvent systems[13,14] and detection was carried out by spraying with silver nitrate[15]. TLC examination (silica gel Ridel-de-Haeneg Seelze-Hannover DGF) was applied using different solvents[16,17], visualisation was carried out using trichloroacetic acid and $FeCl_3$-$K_3Fe(CN0_6$[17].

One spot was only detected corresponding to that of an authentic glucotropaeolin, by applying PC as well as TLC chromatographic techniques. Furthermore, the compound was purified using sheets of Whatman No. 3 MM with n-BuOH- EtOH-H_2O (4:1:4 v/v) (upper phase) for 2 developments each for 20 hrs. at 25°C. The chromatograms were then dried and the strips locating the glucosinolate were cut off, reduced to small pieces, extracted with MeOH-H_2O (30:70 v/v) and evaporated to dryness under reduced pressure.

The tetraacetate derivative of the purified glucosinolate; prepared in the usual manner; melted at 188°C as reported[18].

Preparation of myrosinase:

Myrosinase was prepared by a modification of the procedure of Neuberg and Wagner[19] from seeds of *Sinapis alba* and its activity varified on singirin.

Enzymatic hydrolysis: About 1 mg of the purified glucosinolate was dissolved in 20 ml H_2O. Thereafter, few crystals of ascorbic acid were added, and the solution was buffered to pH 5.6 using citrate buffer. The mixture was treated with the dry enzyme (10 mg.) and left at room temperature (28°C) for 18 hr. Glucose was detected by PC[20], the SO_4^{-2} by $BaCl_2$. Elementary sulfur was detected also in the hydrolysate[21]. The hydrolysate mixture was subjected to steam distillation and the oil was salted out by addition of NaCl then was extracted with chloroform. A yellow pungent oil (0.5 gm) was obtained after evaporation of chloroform *in vacuo* at 40°C. The oil was purified by dissolving in chloroform (200 ml) and passed through silica gel (Griffin and George limited 50-100 mesh) column 2.4 x 30 cm.), the eluant was evaporated *in vacuo*. The oil was then subjected to GC-Ms, IR and NMR.

Preparation of benzyl thiourea: 0.3 gm of the above oil was dissolved in 95% EtOH (1 ml) and treated with the same volume of 25% NH_4OH solution. After warming this mixture on a water bath until the exothermic reaction set in , it was left to cool until the thiourea crystallised out. After recrystallisation from EtOH-H_2O it melted at 165°C, reported 165[22]. The prepared thiourea possesses the same R_f as an authentic benzyl thiourea in different solvents[23,24] using Grote's[25] and iodine azide starch[20] as spraying reagents.

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