IDENTIFICATION OF PHENOLIC ACIDS FROM *ZANTHOXYLUM ZANTHOXYLOIDES* ROOT BY GC-MS

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SUMMARY

A diethylether extract of Zanthoxylum zanthoxyloides yielded the free phenolic acids. The extract was purified by Sephadex ion exchange resins (SP–C25 cationic and DEAE–A25 anionic exchangers in series). p–OH benzoic–, vanillic–, syringic–, ferulic– and caffeic acid were identified from the purified extract by GC–MS. The occurrence of syringic–, ferulic– and caffeic acid is being reported for the first time in this genus.

INTRODUCTION

In 1971, El-Said et al (1) reported that the aqueous extract of *Z. zanthoxyloides* root ("orin ata") prevented the lysis of red blood cells. The other plant materials which were similarly tested did not have this property. During the same year, Sofowora and Isaacs (2) reported that the water extract possessed anti-sickling effects. A water extract was extracted with diethylether, and the diethylether fraction was evaporated to a small volume *in vacuo*, and subjected to PTLC (si gel G); the band at the origin (R_f 0.00-0.05) possessed the antisickling activity (3) and its analysis gave 2-OH methylbenzoic–, o–, m– and p–OH benzoic–, vanillic–, tropic acid, and a few fatty acids (4).

The present communication is the first to report the presence of syringic–, caffeic– and ferulic acid in this root, but their antisickling activity data are not yet available.

MATERIALS AND METHODS

Plant material:

The roots of *Zanthoxylum zanthoxyloides* (Lam.) Waterm. (Rutaceae) were collected on Gbongan–Iwo road in Oyo State of Nigeria; authenticated in the herbarium of the Botany Department of the University of Ife, Nigeria. They were chopped, dried and powdered before use.

Other marterials used:

Sephadex ion exchangers (Pharmacia), authentic phenolic acids (Fluka), GC/MS apparatus (Ribermag R10–10B) using electron impact at 70 ev.

Extraction and purification

0.5 kg was extracted for 24 h with diethylether which was removed *in vacuo*. The residue was suspended in a few ml of water and placed on top of a Sephadex (SP–C25, H^+ form, 10cm x 3cm I.D.) column. The neutral and acidic compounds were eluted with 500 ml water which was reduced to 30 ml and again fractionated on a Sephadex DEAE–A25 (acetate form, 15cm x 3cm I.D.) column. Phenolic compounds without carboxylic acid groupings were eluted with 0.0IM NH_4OAC while phenolic acids were eluted with 0.IN HCL. The latter fraction was extracted four times with an equal volume of ethylacetate, the extracts were bulked and evaporated to dryness. The residue was dissolved in ethanol.

GC/MS ANALYSIS

A suitable aliquot from the above solution was taken and dried completely. 100ml dry pyridine containing dodecane as internal standard followed by 100ml BSTFA with 1 % TMCS were added and the mixture was heated at 90°C for 30 min. 2ml of this solution were injected into the GC/MS apparatus equipped with a 1.5m x 2mm I.D. metal column filled with chromosorb G HP100/120 (5 % OV-101). Temperature programming from 120° to 280° at 6°/min. Nitrogen flow at 20 ml/min. Individual GC peaks (fig. 1) were selected for mass spectrometry (fig. 2).

RESULTS AND DISCUSSION

The gas chromatogram of the trimethylsilyl derivatives of the phenolic acid fraction is shown in fig. 1. At least fifteen different compounds were clearly detected, but only the following phenolic acids : p–OH benzoic–, vanillic–, syringic,– caffeic– and ferulic acid were identified by GC/MS. Vanillic– and syringic acid appeared to be the two major phenolic acids in this root as indicated by the chromatogram, while ferulic– and cafeic acid were detected in small amounts. Their identity was confirmed by the mass spectra of the trimethylsilyl derivatives (fig. 2). Detailed fragmentation patterns of the silylated phenolic acids can be found in the literature (5, 6). The following fragment ions are characteristic : $M^+ - 15$, $M^+ - 59$ and $M^+ - 89$ (table 1). The identities of these compounds were further confirmed by comparison with the GC/MS data of authentic compounds.

Table 1: Characteristic fragment ions for identifying phenolic acids in *Zanthoxylum zanthoxyloides.*

Phenolic Acid	Fragment ions m/e (rel. int.*)			
	M^+	M^+-15	M^+-59	M^+-89
p–OH benzoic	282 (8.0)	267 (33.4)	223 (25.8)	193 (26.7)
Vanillic	312 (43.5)	297 (71.9)	253 (35.2)	223 (56.1)
Syringic	342 (28.9)	327 (38.9)	283 (6. 6)	253 (18.6)
Ferulic	338 (25.1)	323 (15.5)	279 (2.0)	249 (13.1)
Caffeic	396 (14.6)	381 (2. 4)	337 (–)	307 (1.2)

*The intensities are indicated as percentages relative to the base peak which has m/e 73, taken as 100%.

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