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Identification of methyl salicylate as the principal volatile component in the methanol extract of root bark of *Securidaca longepedunculata* Fers

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Securidaca longepedunculata **Fers (Polygalaceae) is commonly used as a medicine in many parts of Africa and shows promise for protecting stored grain against insect pests. Analysis of a methanol extract of the root bark by gas chromatography linked to mass spectrometry (GC/MS) showed a major component accounting for over 90% of the volatile material. This was identified as methyl 2-hydroxybenzoate (methyl salicylate) by comparison of the GC retention times and mass spectrum with those of synthetic standards. This conflicts with an earlier report that the major component is methyl 4-hydroxybenzoate. Two minor components had mass spectra characteristic of 2-hydroxybenzoate esters and were identified as methyl 2-hydroxy-6-methoxybenzoate and its benzyl analogue, again conflicting with an earlier report.** **Copyright © 2002 John Wiley & Sons, Ltd.**

KEYWORDS: *Securidaca longepedunculata*; Polygalaceae; methyl 2-hydroxybenzoate; methyl salicylate; methyl 3-hydroxybenzoate; methyl 4-hydroxybenzoate; methyl 2-hydroxy-6-methoxybenzoate; benzyl 2-hydroxy-6-methoxybenzoate; gas chromatography, mass spectrometry

INTRODUCTION

Securidaca longepedunculata Fers (Polygalaceae) is commonly used as a medicine in many parts of Africa for the treatment of rheumatic conditions, fever, headache and various other inflammatory conditions.[1–3] Dried powdered roots are also used as a pest control agent in storage, and methanol extracts of the roots have potential as a protectant against insect pests in stored grain.[4] During work to investigate the active principle(s) in these extracts, we analysed the volatile fraction and found a single major component accounting for over 90% of volatile material. Costa *et al.*[5] claimed that this was methyl 4-hydroxybenzoate based on mass spectral data, but in this paper we present evidence that in our material, and probably that of Costa *et al.*,[5] the major component is methyl 2-hydroxybenzoate (methyl salicylate). Furthermore, at least two of the minor components have structures related to the latter and are probably not as identified by Costa *et al.*[5]

EXPERIMENTAL

Roots of *S. longepedunculata* were collected from the Tamale area in Ghana, West Africa in 1999. The bark from the roots was removed (100 g), ground to a fine powder and extracted with methanol (1000 ml) for 24 h at 20 °C. The extract was filtered and concentrated under vacuum at 30 °C to give a solid (25 g). A sample (5 g) of this was redissolved in methanol (15 ml), mixed with silica gel (Merck silica gel 60, 0.040–0.063 mm, 5 g) and dried under vacuum at 30 °C. The dried methanol extract adsorbed on silica gel was loaded on a silica gel column (10 cm × 5 cm i.d.) and the column was eluted with chloroform–methanol (100 : 1; 100 ml). The eluate was concentrated under vacuum to 10 ml and an aliquot (3 ml) of this fraction was dissolved in hexane (27 ml).

The sample was analysed by GC/MS using a Carlo Erba Mega instrument fitted with a fused-silica capillary column (25 m × 0.25 mm i.d.) coated with CPSil5CB (methyl silicone) (Chrompack, Middleburg, The Netherlands) connected directly to a Finnigan ITD 700 ion trap detector (trap and interface temperature 230 °C) operated in the electron impact ionization mode (scanning mass range m/z 40–350). The injector temperature was 200 °C, the carrier gas was helium (0.5 kg cm^{-2}) and the oven temperature was held at 60 °C for 2 min and then increased to 230 °C at 6 °C min^{-1}. Injection was performed in the split mode (~50 : 1; 1 µl), and retention data are presented as Kováts retention indices[6] (KI) relative to the retention times of normal hydrocarbons.

Retention data were also recorded by GC with flame ionization detection (FID) using a fused-silica capillary column (25 m × 0.32 mm i.d.) coated with CPWax52CB (Carbowax equivalent) (Chrompack) operated under conditions identical to those above.

Synthetic methyl esters of 2-, 3- and 4-hydroxybenzoic, 4-methyl-2,6-dihydroxybenzoic and 3-, 4- and 5-methoxy-2-hydroxybenzoic acids were obtained from Aldrich (Gillingham, Dorset, UK) and used as supplied. The

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methyl and benzyl esters of 2-hydroxy-6-methoxybenzoic acid were obtained by reaction of the acid (Aldrich) with the appropriate alcohol in dichloromethane in the presence of 4-dimethylaminopyridine and *N*,*N*′-dicyclohexylcarbodiimide.[7] The products were purified by flash chromatography on silica gel and distillation and their structures were confirmed by NMR spectroscopy.

RESULTS

The GC/MS total ion chromatogram of the methanolic extract of root bark of *S. longepedunculata* run on the non-polar GC phase is shown in Fig. 1. The major component (A) constituted ~93% of the volatile material observed and was estimated to be present at ~3 mg g^{-1} of bark. The mass spectrum of this component is shown in Fig. 2(a). Search of the NBS library supplied with the ITD spectrometer suggested it was methyl 2-hydroxybenzoate with a fit of 934. The methyl 3- and 4-hydroxybenzoates gave fits of 892 and 891, respectively. The mass spectra of the three synthetic isomers run under the same conditions are shown in Fig. 2(b)–(d). The mass spectra of the natural compound [Fig. 2(a)] and methyl 2-hydroxybenzoate [Fig. 2(b)] are essentially identical and clearly different from those of the other two isomers [Fig. 2(c) and (d)]. The former show a molecular ion (m/z 152) with ions corresponding to loss of methanol (M – 32; m/z 120) followed by loss of the carbonyl group (M – 32 – 28; m/z 92). In contrast, the latter two spectra show loss of methoxy from the molecular ions (M – 31; m/z 121) followed by loss of the carbonyl (M – 31 – 28; m/z 93). This is consistent with the effect of internal hydrogen bonding between the vicinal hydroxyl and ester groupings in the 2-hydroxy isomer which is not present in the 3- and 4- isomers.[8]

Comparison of the GC retention times of the three isomers on non-polar and polar phases (Table 1) confirmed that the

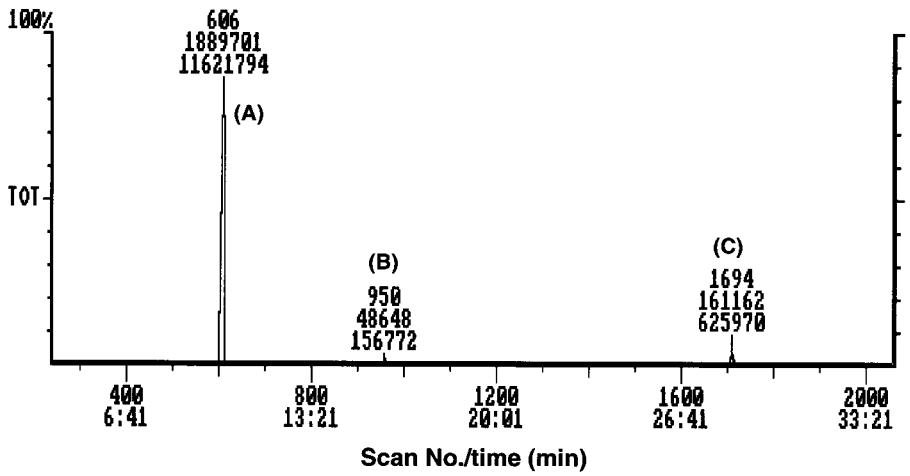

Figure 1. GC/MS analysis of methanol extract of *S. longepedunculata* (CPSil5CB).

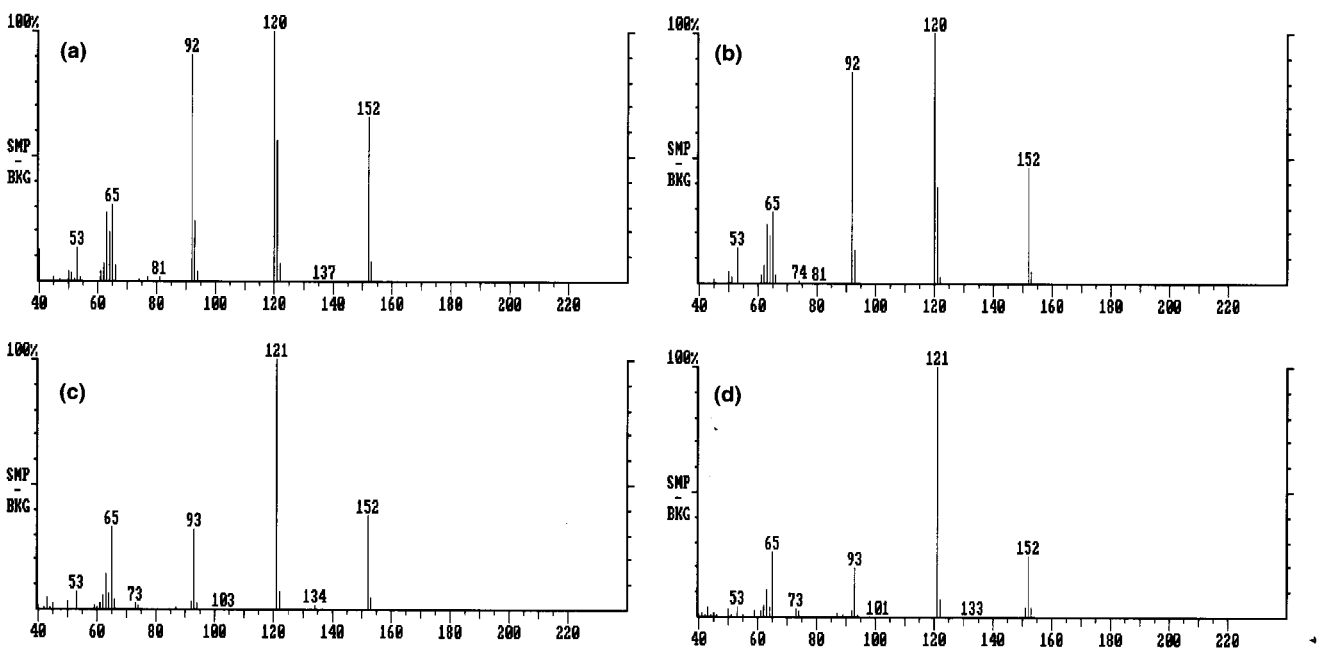

Figure 2. Mass spectrum of (a) main volatile compound from roots of *S. longepedunculata*, (b) methyl 2-hydroxybenzoate, (c) methyl 3-hydroxybenzoate and (d) methyl 4-hydroxybenzoate.

natural compound (A) had the same retention times as methyl 2-hydroxybenzoate [Fig. 3 (I)] which were clearly different from those of the 3- and 4-isomers on both phases. The internal hydrogen bonding present in the 2-hydroxy isomer gave this much shorter retention times than the other two isomers on both phases.

In our sample, two other significant minor components, (peaks B and C in Fig. 1) were detected at KI 1403 (~1% of volatiles) and KI 2045 (~5% of volatiles), respectively, on the non-polar CPSil5CB column. The mass spectra of these two components are shown in Fig. 4(a) and (b),

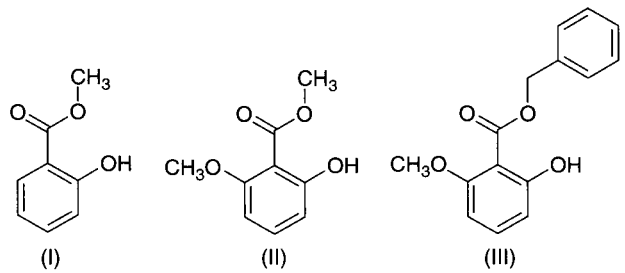

Figure 3. Proposed structures of components of methanol extract from roots of *S. longepedunculata*.

Table 1. Kováts retention indices (KI) of main volatile components in methanol extract of roots of *S. longepedunculata* and synthetic hydroxybenzoates on non-polar CPSil5CB and polar CPWax52CB columns

	GC phase	
Compound	CPSil5CB	CPWax52CB
Component A	1172	1769
Component B	1403	2236
Component C	2045	n.o.[a]
Methyl 2-hydroxybenzoate	1172	1768
Methyl 3-hydroxybenzoate	1384	2845
Methyl 4-hydroxybenzoate	1420	3015
Methyl 2,6-dihydroxy-4-methylbenzoate	1474	2360
Methyl 2-hydroxy-3-methoxybenzoate	1417	2239
Methyl 2-hydroxy-4-methoxybenzoate	1426	2217
Methyl 2-hydroxy-5-methoxybenzoate	1400	2151
Methyl 2-hydroxy-6-methoxybenzoate	1403	2235
Benzyl 2-hydroxy-6-methoxybenzoate	2046	n.o.[a]

[a] Not observed.

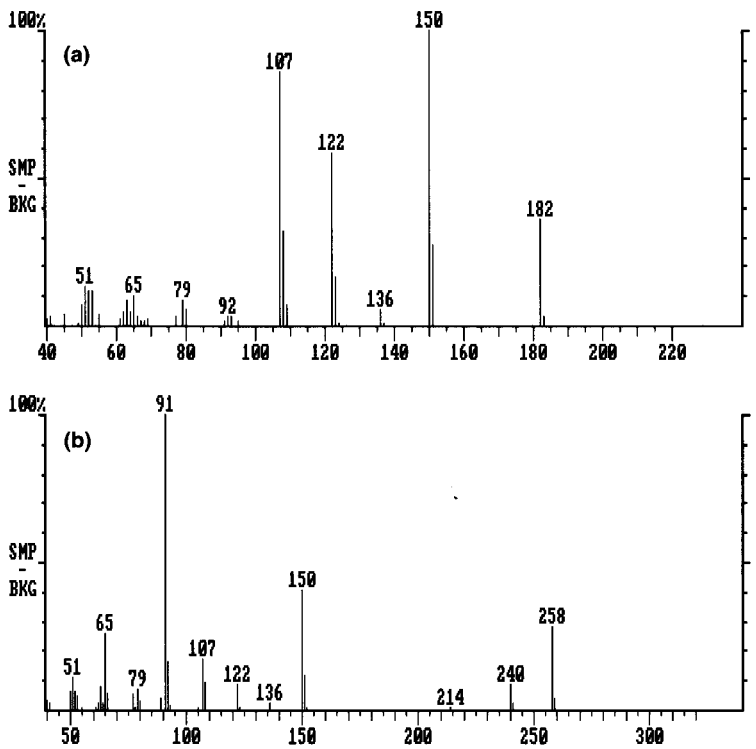

Figure 4. Mass spectra of (a) minor component B and (b) minor component C from roots of *S. longepedunculata*.

respectively. The first (B) shows a molecular ion (m/z 182) with loss of methanol (M – 32; m/z 150) followed by loss of carbonyl (M – 32 – 28; m/z 122) analogous to the fragmentation pattern of methyl 2-hydroxybenzoate [Fig. 2(b)]. These data suggest that the compound might be a methyl 2-hydroxybenzoate with additional oxygen and methyl functions. Comparison of the GC retention times and mass spectra with those of the methyl esters of 2,6-dihydroxy-4-methylbenzoic and 2-hydroxy-3-, 4-, 5- and -6-methoxybenzoic acids showed that the data for the natural compound (B) were identical with those of methyl 2-hydroxy-6-methoxybenzoate [Fig. 3 (**II**)] and different from those of the other isomers examined. The natural and synthetic compounds showed co-chromatography on both GC phases.

The mass spectrum of the second minor component (C) [Fig. 4(b)] showed a molecular ion at m/z 258 with fragmentation indicating loss of benzyl alcohol (M – 108; m/z 150) followed by loss of carbonyl (M – 108 – 28; m/z 122). As with compounds **I** and **II**, the loss of even-mass fragments suggests the presence of a 2-hydroxybenzoate ester, and the data were consistent with those expected for the benzyl analogue of compound **II**, i.e. benzyl 2-hydroxy-6-methoxybenzoate [Fig. 3 (**III**)]. This compound was synthesized and shown to co-chromatograph with the natural component (C) on the CP Sil5CB column and to have an identical mass spectrum. The compound does not elute from a CPWax52CB column, as indicated in Table 1.

DISCUSSION

The GC and MS data indicate that the major volatile component of our methanol extract of the root bark of *S. longepedunculata* is methyl 2-hydroxybenzoate (methyl salicylate (**I**)). This is consistent with the report of Oliver-Bever[1] and the presence of this compound in many other Polygalaceae.[9] However, this identification conflicts with the report of Costa *et al.*[5] who concluded the major component in their extract was methyl 4-hydroxybenzoate.

The mass spectrum shown by Costa *et al.*[5] is actually different from that of any one of the methyl hydroxybenzoate isomers, having ions at m/z 152, 121 and 92 (Fig. 2(a) in Costa *et al.*[5] cf. Fig. 2 in this paper) although the authors claimed a good match with that of the 4- isomer. These authors used an OV-17 GC column, but did not give relative retention data. We were not able to analyse our extract on an OV-17 column, but the short retention time of 7.5 min shown by Costa *et al.*[5] would suggest that the major component in their extract was also methyl 2-hydroxybenzoate.

Costa *et al.*[5] identified nine other components of the extract on the basis of their mass spectra. We detected two significant minor components in our extract, and their mass spectra are identical with those of components 2 (molecular ion m/z 182) and 8 (molecular ion m/z 258) in Costa *et al.*[5] Both showed the highly characteristic fragmentation patterns of 2-hydroxybenzoate esters. In our extract, the former of these had a GC retention time and mass spectrum identical with those of methyl 2-hydroxy-6-methoxybenzoate (**II**), and not methyl 4-hydroxy-3-methoxybenzoate as reported by Costa *et al.*[5] The GC retention time and mass spectrum of the second minor component were identical with those of the benzyl analogue, benzyl 2-hydroxy-6-methoxybenzoate (**III**), and not that of the phenyl 3-amino-5-nitrobenzoate suggested by Costa *et al.*[5]

Work is in progress on the full identification of minor components in *S. longepedunculata* root bark and evaluation of their anti-insect activity, and results will be reported elsewhere. Methyl 2-hydroxybenzoate and the corresponding acid, but not the 4-isomers, are becoming well established as plant stress signals countering pest attack[10] through regulation of gene expression.[11] 2-Hydroxy-6-methoxybenzoic acid and derivatives have only occasionally been reported as plant chemicals.[12–14] However, after completion of this work, a recent report[15] was found confirming the presence of methyl 2-hydroxybenzoate and methyl 2-hydroxy-6-methoxybenzoate in root bark of *S. longepedunculata*, but not the benzyl ester.

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