

Research Article

Tentative detection of some alkyl coumarates and alkyl ferulates in *Ipomoea carnea* subsp. *fistulosa* by HRESIMS and comparison of these compounds among Convolvulaceae plants

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ABSTRACT: Three alkyl coumarates (hexadecyl coumarate, eicosyl coumarate and docosyl coumarate) and five alkyl ferulates (tetradecyl ferulate, pentadecyl ferulate, heptadecyl ferulate, octadecyl ferulate and nonadecyl ferulate) could be tentatively detected in the partially purified HPLC fraction of *Ipomoea carnea* subsp. *fistulosa* by high resolution electrospray ionization mass spectroscopy (HRESIMS). This is in addition to octadecyl *p*-coumarate whose detailed structure elucidation, antifungal activity and isomerization have already been reported. The compounds were compared with alkyl coumarates and alkyl ferulates reported from other Convolvulaceae plants. It was found that octadecyl *p*-coumarate is the most frequently reported alkyl coumarate in the family. It was also observed that while the number carbon atoms in the alkyl groups of alkyl coumarates reported from Convolvulaceae is always even, in the case of alkyl ferulates, number of carbon atoms of the alkyl moiety can either be odd or even.

KEYWORDS: *Ipomoea carnea* subsp. *fistulosa*, alkyl coumarates, alkyl ferulates, Convolvulaceae, HRESIMS

INTRODUCTION

Ipomoea carnea subsp. *fistulosa* (Convolvulaceae) is a plant native to South America and is also sparsely distributed in India and Bangladesh. Anticancer and anti HIV properties have been attributed to the extractives of the plant.^[1] A recent communication from this institute reported bioassay monitored isolation and characterization of the chief antifungal fraction of the plant as a mixture of (*E*) and (*Z*) isomers of octadecyl *p*-coumarates.^[2] A detailed study on the delineation of NMR signals of *E* and *Z* isomers of octadecyl *p*-coumarates has also been reported.^[3] Methyl *p*-coumarate, octyl *p*-coumarate and dodecyl *p*-coumarate have also been reported recently from the plant.^[4] A literature survey found further reports on alkyl coumarates including octadecyl *p*-coumarates and alkyl ferulates in other plants of the Convolvulaceae family.^[5–11]

Co-occurrence of alkyl coumarates and alkyl ferulates in Convolvulaceae has been established.^[12]

Alkyl coumarates exhibit wide range of biological activities. Antibacterial,^[13] phytotoxic,^[14] insect resistance,^[6] melanin formation inhibition,^[15] antioxidant,^[16] DNA polymerase inhibition,^[17] DNA topoisomerase inhibition, human cancer cell growth inhibition^[18] and antifungal^[2] activities have all been shown to be induced by alkyl coumarates. Antioxidant properties have been attributed to alkyl ferulates.^[19]

Structure elucidation of naturally occurring compounds usually involves extensive chemical and spectral studies for which sufficient amounts of the compound have to be obtained in pure form. This is extremely difficult if the compounds are present in plants only in trace amounts. However, in high resolution electrospray ionization mass spectroscopy (HRESIMS), the molecular formula of a molecular ion can be determined directly by comparison of the ion masses at high resolution with possible composition using accurate masses of individual isotopes. Since the electrospray method is a soft ionization procedure, fragmentation of ions is less compared to electron impact method. Once the molecular formula is obtained,

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it is possible to compare this with those of compounds already isolated from the plant or related species and arrive at logical inferences leading to the chemical nature of compounds. For this purpose, the mass accuracy of the compounds should be within reasonable limits. Mass accuracy (M_a) is given by the formula

$$M_a = \frac{(\text{Calculated mass} - \text{observed mass}) \times 10^6}{\text{Calculate mass}} (\text{ppm})$$

While mass accuracy is always preferred to below 5 ppm,^[20] values up to 58 ppm are often reported for natural compounds.^[21] Although such inferences can be considered as tentative only, they can be of importance for further research particularly from chemotaxonomic point of view.

From this standpoint, tentative detection of three alkyl coumarates and five alkyl ferulates in the partially purified HPLC fraction of *Ipomoea carnea* subsp. *fistulosa* by HRESIMS is being reported in this paper. This is in addition to octadecyl *p*-coumarates whose detailed structure elucidation by extensive spectral studies has already been reported.^[2] The compounds are being compared with alkyl coumarates and alkyl ferulates reported in different plants of Convolvulacea family.

MATERIALS AND METHODS

Details of bioassay monitored isolation, HPLC purification, structure elucidation by spectral methods, isomerization and antifungal activity of octadecyl *p*-coumarates have been reported in the earlier paper.^[2] Column chromatography of the crude ethyl acetate extractive over silica gel with hexane-ethyl acetate mixture (7:3) gave a fraction which was subjected to HPLC. HPLC purification was performed using a Waters HPLC system (515 pump, 7725 Rheodyne injector, Waters 2487 Dual λ absorbance detector) with conditions as follows: Column Prep Nova Pak HR Silica 7.8 \times 300 mm, flow rate 1 ml/min, UV detection at 254 nm, eluent CH₂Cl₂. The HPLC fraction corresponding to $t_r = 48$ minutes was collected. After the first stage of purification, it was further purified once more. This fraction after removal of solvent was subjected to TLC bioassay^[21] using *Cladosporium cucumerinum*. This fraction was also subjected to HRESIMS. The spectrum was obtained on Micromass Q-TOF apparatus.

RESULTS AND DISCUSSION

The fraction obtained after two stages of purification exhibited antifungal activity against the spore germination of *Cladosporium cucumerinum* by TLC bioassay^[21] at a dose of 0.3 mg. From the HRESIMS spectrum (Figure 1), it was clear

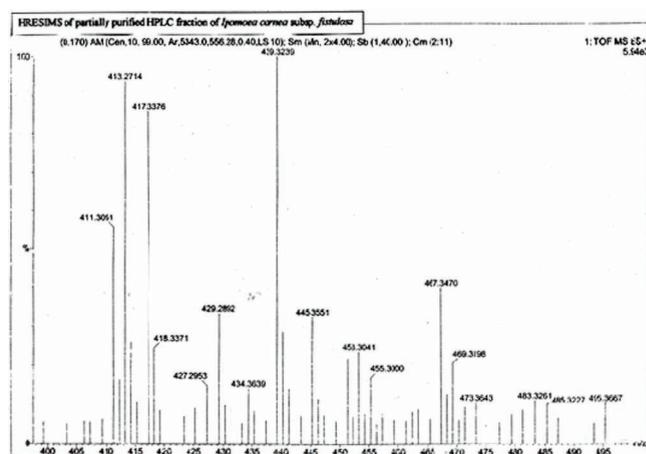
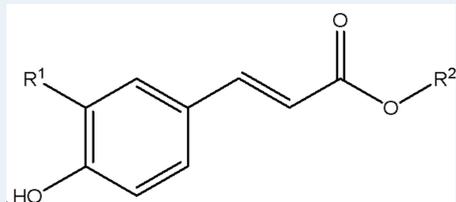


Figure 1. HRESIMS of partially purified HPLC fraction of *Ipomoea carnea* subsp. *fistulosa*

that the fraction did not correspond to any single compound. Thus it was subjected to three more stages of HPLC purification (total five stages). Detailed study of this fraction using C, H analyses, HRESIMS, CIMS, IR, ¹H NMR and ¹³C NMR revealed that it was octadecyl *p*-coumarate obtained in pure form.^[2] Once the structure of the major active principle was identified as octadecyl *p*-coumarates by extensive spectral studies, it was possible to arrive at logical inferences about other peaks in HRESIMS of the partially purified fraction after two stages in relation to published literature on alkyl coumarates and alkyl ferulates. It was found that the most of the peaks corresponded to compounds reported from other *Ipomoea* species or structurally related to octadecyl *p*-coumarates.

In this way, hexadecyl coumarate, tetradecyl ferulate, pentadecyl ferulate, heptadecyl ferulate, eicosyl coumarate, octadecyl ferulate, nonadecyl ferulate and docosyl coumarate could be tentatively detected. Details are given in Table 1. Since this paper deals with only a tentative detection of alkyl coumarates and alkyl ferulates of the plant, mass accuracy values up to 45 ppm were considered valid. Because these compounds are structurally related to octadecyl *p*-coumarates, they may be exhibiting isomerization as reported in the case of octadecyl *p*-coumarates.^[2] However, structures of only (*E*) isomers are given in Table I. Further, position of the hydroxyl group, methoxyl group and straight chain nature of the alkyl groups are presumed based on the structures of majority of the compounds isolated from Convolvulacea.

The results are being compared with alkyl *p*-coumarates and alkyl ferulates reported from other plants of Convolvulacea family in Table 2. It may be noted that octadecyl *p*-coumarate is the most widely reported alkyl *p*-coumarate. It is also noteworthy that while number carbon atoms in the alkyl groups of alkyl coumarates reported from Convolvulacea

Table 1: Alkyl coumarates and alkyl ferulates tentatively detected in *Ipomoea carnea* subsp. *fistulosa*

No.	Obs. Mass -Na	Mol. formula	Calc. mass	M _a	Compound	R ¹	R ²
1	388.3153	C ₂₅ H ₄₀ O ₃	388.2978	45	Hexadecyl Coumarate	H	
2	390.2816	C ₂₄ H ₃₈ O ₄	390.2770	12	Tetradecyl Ferulate	OCH ₃	
3	404.3055	C ₂₅ H ₄₀ O ₄	404.2927	32	Pentadecyl Ferulate	OCH ₃	
4	416.3341	C ₂₇ H ₄₄ O ₃	416.3291	12	Octadecyl Coumarate*	H	
5	432.3102	C ₂₇ H ₄₄ O ₄	432.3240	32	Heptadecyl Ferulate	OCH ₃	
6	444.3572	C ₂₉ H ₄₈ O ₃	444.3604	7	Eicosyl Coumarate	H	
7	446.3300	C ₂₈ H ₄₆ O ₄	446.3396	22	Octadecyl Ferulate	OCH ₃	
8	460.3373	C ₂₉ H ₄₈ O ₄	460.3553	39	Nonadecyl Ferulate	OCH ₃	
9	472.3769	C ₃₁ H ₅₂ O ₃	472.3917	31	Docosyl Coumarate	H	

M_a = Mass accuracy *Confirmed compound**Table 2: Alkyl coumarates and alkyl ferulates reported from Convolvulaceae**

No.	Plant	Coumarates/Ferulates	Alkyl groups	Reference No.
1	<i>Ipomoea carnea</i> subsp. <i>fistulosa</i>	Coumarates	Methyl, Octyl and dodecyl. Hexadecyl, octadecyl, eicosanyl and docosyl	4 2 and the present study
		Ferulates	Tetradecyl, pentadecyl, heptadecyl, Octadecyl and nonadecyl	Present study
2	<i>Ipomoea batatas</i>	Coumarates	Hexadecyl, octadecyl and eicosyl	6
		Ferulates	Hexadecyl, heptadecyl and octadecyl	11
3	<i>Ipomoea digitata</i>	Coumarates	Octadecyl	8
4	<i>Argyrea populifolia</i>	Coumarates	Octadecyl	5
5	<i>Cascutta chinensis</i>	Coumarates	Octadecyl	10
6	<i>Merremia tuberosa</i>	Coumarates	Hexadecyl and octadecyl	1

is always even, in the case of alkyl ferulates, number of carbon atoms of the alkyl moiety can either be odd or even. The report on the presence of alkyl coumarates and alkyl ferulates in several Convolvulaceae plants may be of

interest from the chemotaxonomic point of view. The wide range of biological activities attributed to alkyl coumarates and alkyl ferulates will be of interest from the pharmacological use of Convolvulaceae plants also.

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