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ENOL ESTERS OF CAFFEIC ACID IN SEVERAL GENERA OF THE LABIATAE

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Key Word Index—Labiatae; tissue culture; phenylpropanoid pigments; caffeic esters of enols; metal chelation; fungotoxicity.

Abstract—The (*Z*, *E*) and (*E*, *E*) isomers of the enol ester formed by condensation of dopaldehyde with caffeic acid, which had previously only been reported from two plant sources, have been found in the foliage and in cell cultures of seven of eight species (of several genera) of the Labiatae that were screened. The compounds were not detected in several species from other families of higher plants. These esters efficiently formed complexes with Fe (II) and were potent fungicides towards *Cladosporium herbarium*.

INTRODUCTION

Following our discovery that callus cultures of *Lavandula angustifolia* Mill (subsp. *angustifolia* ≡ *L. officinalis* Chaix ≡ *L. vera* DC; Labiatae) appeared to secrete an intensely blue pigment into the supporting agar, we demonstrated that the coloration resulted from complexing of Fe(II) in the nutrient medium by the (*Z*,*E*) and (*E*,*E*) isomers of the enol ester of dopaldehyde with caffeic acid [2-(3,4-dihydroxyphenyl) ethenyl esters of 3-(3,4-dihydroxyphenyl)-2-propenoic acid] [1]. These yellow compounds (**1**, **2**; Fig. 1) hereafter termed 'pigments' could be found *in callo* but were predominantly released into the culture medium; they could not be detected (< 1 ppm) in the parent plants. The (*Z*,*E*)-isomer had previously been reported in the foliage of *Plectranthus caninus* Roth (≡ *Coleus spicata*; Labiatae) [2], but to our knowledge only one other example exists of a related type of enol ester as a natural product [3].

We here report studies to delimit the distribution of these interesting pigments, and to indicate their possible biological significance.

RESULTS AND DISCUSSION

Distribution of pigments

Samples of pigments were available from our previous investigation and we confirmed the structures and were able to assign all the resonances in the ^{1}H NMR spectrum by use of the COSY technique at 400 MHz: previously, certain of the assignments and couplings had been uncertain. Adopting these compounds as standards, we screened the eight Labiatae species listed in Table 1 using foliage (stem and leaves) and in some examples callus and fine cell suspension cultures. The cultures were easily established—the conditions and properties are listed in the Experimental Section—and as the assays were made after at least six (and more usually 10) passages, any carry-over of components of the explant must have been negligible. In addition, all field-grown plants and some cultures were qualitatively screened for the occurrence of rosmarinic acid (**3**) which is presumably related biosynthetically but is, in contrast, known to be widely distributed in the Labiatae.

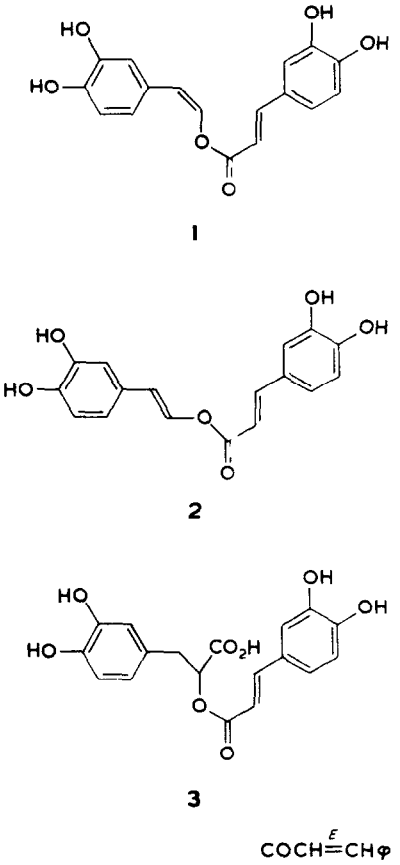

Fig. 1.

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Table 1. Occurrence of yellow pigments in Labiatae

Species	RA*	% Pigments (1, 2)†		
		Foliage	Callus	Suspension
Lavandula angustifolia L.¶	+	0.00	0.21 (3:1)‡	0.01 (4:1)
Plectranthus caninus Roth.	+	0.02 (2:1)	0.04 (1:1)	–
Rosmarinius officinalis L.	+	0.03 (6:1)	trace§	–
Mentha spicata cv. Lacinata.	+	0.01 (**1** only)	0.01 (4:1)	0.02 (3:1)
Mentha longifolia (L) Huds.	+	trace	>0.01 (3:1)‖	–
Salvia officinalis L.	+	0.04 (9:1)	trace	–
Hyssopus officinalis L.	+	trace	0.00	–
Teucrium fruticans L.	–	0.00	0.00	–

*Rosmarinic acid: qualitative assay (*ca* 1×10^{-3}% wt/fr. wt detectable).
†% wt/fr. wt of **1** + **2**; s.e. *ca* ± 0.002 actual value.
‡Also secreted into medium.
‖Imprecise assay because of accidental weighing errors.
¶Similar results were obtained with the cultivars Hidcote and Munstead Dwarf.
§Qualitative assay (spot on TL-chromatogram), *ca* 1×10^{-6} to 1×10^{-3}% wt/fr. wt. present.

All plants and/or cultures except *T. fructicans* were found to contain detectable and sometimes appreciable quantities of the pigments. The values quoted are weight per fresh weight, but because of the relatively high water content of cultures compared with that of their parent plants, a dry weight comparison would produce a much more favourable pattern of accumulation for the former. The parent plants also contained a plethora of the lower terpenoids and other secondary metabolites in the extractable oils but in no example was more than traces (*ca* 10^{-6}% wt/fresh wt; if any) of these compounds detected in the extracts from tissue cultures even when a two-phase trapping system using the non-toxic triglyceride Miglyol [4, 5] was employed for cell suspensions. Others, however, have found appreciable accumulations of lower terpenoids in their culture lines of *M. spicata* cf. ref. [6]. The pigments were the main components of the extracts from the cultures and the accumulations achieved were excellent in comparison with those typically reported for secondary metabolites from such tissue. The other characterized components from the extracts of cultures were phytosterols: thus callus of *L. angustifolia* contained isofucosterol, sitosterol and stigmasterol (0.01, 0.03 and 0.005% wt/fresh wt) and *M. spicata* callus accumulated lower levels of the last two (0.001 and 0.0003%). Rosmarinic acid was detected in all the parent species except *T. fruticans* but was *not* found in the two cultures (callus of *L. angustifolia* and *P. caninus*) that were screened.

The occurrence (in cultures of *L. angustifolia*) of high levels of metabolites that do not accumulate in the parent plant is a situation that has precedents for other species, cf. refs [7–9], and may result from a metabolic block or the loss of control factors in culture leading to magnified production or to accumulation of the pigments. The naturally occurring levels in the field-grown plants of *L. angustifolia* may also be unusually low—cf. the levels in the other members of the Labiatae listed in Table 1. Of the callus culture lines analysed, those from *L. angustifolia* alone excreted the pigments into the medium with the resulting formation of the blue coloration. Attempts to develop similar 'leaky' cultures of *P. caninus* by cold-treatment or irrigation with dimethylsulphoxide [10] failed. It was also demonstrated for *L. angustifolia* Mill. (and also for the *L. angustifolia* cvs 'Hidcote' and 'Munstead Dwarf') that fine cell suspensions, and also cells immobilized on polyurethane foam or in agar matrix films also accumulated the pigments (*ca* 0.01% wt per fresh wt). The products were not now released into the medium but the cell surfaces became intensely blue, and the Fe complex could readily be extracted from the disintegrated cells with dimethylsulphoxide. Such lack of secretion or blue surface pigmentation was never found in any callus line of *L. angustifolia* or other species. An unidentified blue pigment (presumably composed of the complexes reported in the present study) has recently been reported to accumulate in gel-entrapped cells of *L. angustifolia* [11].

We record an investigation of the effects of type of hormone and sugar-levels on the accumulation of pigments and steroids in cultures of *M. spicata* in Table 2. Similar, but less extensive results were obtained for *L. angustifolia*. Callus and suspensions accumulated approximately equivalent levels of pigments irrespective of the hormones administered, but there was a pronounced and reproducible effect of increased sugar levels on the quantities of pigments. An effect similar to the latter has been reported for the accumulation of rosmarinic acid in cultures of *C. blumei* [12]. The optimum rate of growth of both our callus lines when sucrose was the additive occurred on 2% sucrose and the cultures obtained on 8% sucrose appeared equally healthy, but those established on the medium with 0.2% sucrose were brown, rubbery and necrotic.

When pigments occurred in field-grown plants they comprised up to 10% of the extractable oil and were very obvious (by virtue of their colour) on chromatograms. These compounds may have been hitherto undetected (despite repeated analysis of the essential oils from these species) because they are steam-involatile and also being acid-labile may not have survived conventional maceration and solvent extraction procedures. Such apparently widely distributed compounds, also expressible in culture, could have potential use as taxonomic markers for

Table 2. Effect of hormones and sugar levels on pigment production in cultures of *Mentha spicata*

Type	Additive	% Pigment (1, 2)	$10^3 \times$ (% Sitosterol)†	Growth rate§
Callus	2,4-D (6 mg/l)*	0.01 (4:1)	1.1	90±15
Callus	NAA (0.5 mg/l)+Kinetin* (0.1 mg/l)	0.01 (3:1)	1.0	82±16
Suspension	2,4-D (6 mg/l)*	0.01 (3:1)	0.6	—
Callus	Sucrose‡ (80 g/l)	0.03 (4:1)	4.0	27±5
Callus	Sucrose (20 g/l)	0.01 (4:1)	3.1	90±15
Callus	Sucrose (2 g/l)	0.00 (−)	1.0	13±4
Callus	Glucose (20 g/l)	0.01 (12:1)	2.9	102±20
Callus	Maltose (20 g/l)	0.01 (9:1)	2.0	116±16

*Additives to MS basal medium + 20 g/l sucrose.
†Stigmasterol was also present at levels 10 to 20% those of sitosterol.
‡For experiments concerning the effects of variation of sugar levels and type, MS medium + 2,4-D (6 mg/l) was employed.
§mg/g/day over 28 day passage.

the family just as other caffeic acid derivatives have been found to have such significance in the Labiatae [13]. The sub-classification of this family is not fully agreed, but our selection of genera does appear to include a range of sub-families [14–17].

We took the opportunity to screen cultures, for which large quantities of biomass were available, from other families for the presence of the pigments, but they could not be detected in *Jasminum officinale* (Oleaceae); *Artemisia vulgaris*; *Tanacetum vulgare* and *T. parthenium* (Compositae); *Anethum graveolens* (Umbelliferae), *Rosa damascena* (Rosaceae); and *Pinus radiata* and *P. pinaster* (Pinaceae).

Biosynthesis and biological significance

The pigments have unusual structures in that the quasi-alcoholic moiety appeared to have reacted in the enol form which is thermodynamically unfavourable. However, a reasonable route to these compounds and the presumably related rosmarinic acid can be devised that avoids this problem, see Fig. 2. The C_6-C_2 unit (**5**) of the molecules is also hypothetically involved in the biosynthesis of the benzylisoquinoline alkaloids where it contributes the benzyl portion of the skeleton. However it is known [18] that the actual biosynthetic precursor in these latter situations is the phenylpyruvic acid **4**: this is the biogenetic equivalent of **5** and the latter probably never exists as a kinetically free species. If **4** also be a precursor in our systems, a reduction–esterification route to rosmarinic acid (**3**) may be posited. Similarly, we can envisage the addition, esterification and elimination sequence to the pigments **1** and **2**, perhaps with the participation of an enzyme with a cysteine or methionine residue at the active site. In this latter pathway, neither a keto nor enol form of the reactant is implicated and the unfavourable thermodynamic situation is circumvented. The esterification step may well involve caffeoyl-CoA cf. [19] and the several steps (adjustable in detail) shown in Fig. 2 could allow for influence on the pathway of

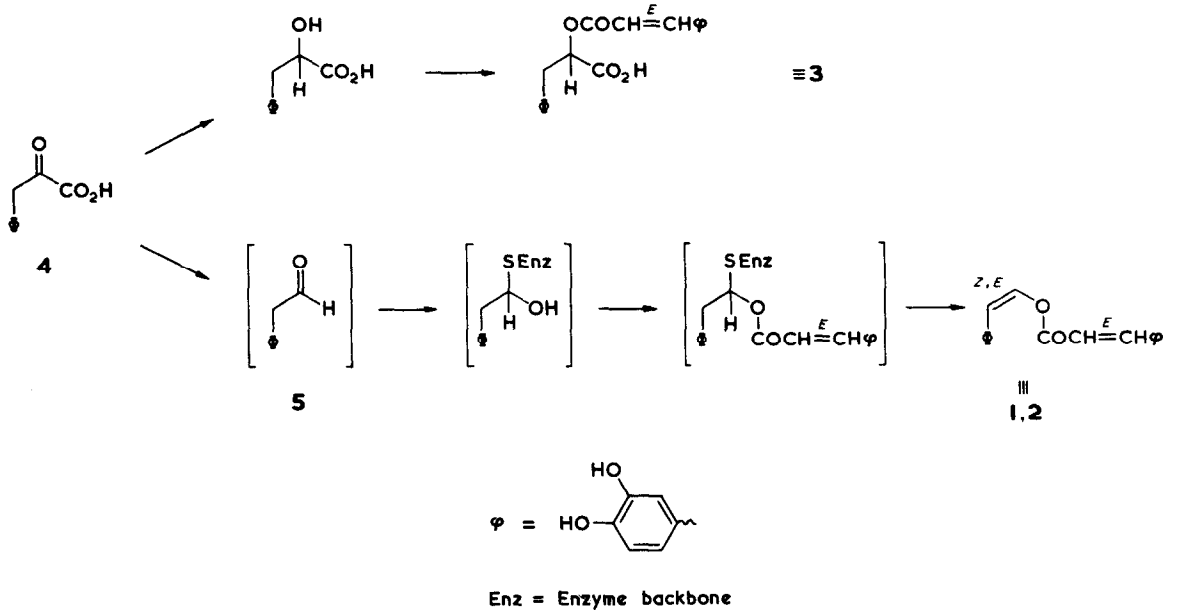

Fig. 2.

additives with redox properties. This may accomodate recent findings that several such additives effect the rate of accumulation of an unidentified blue pigment (presumably **1** and **2** complexed to Fe(II)) in cell suspensions of *L. angustifolia* [20]: these findings are difficult to account for by alterations in the Fe(II) and Fe(III) levels in the medium.

The spectacular complexing properties of **1** and **2** towards Fe(II) prompted a suggestion [1] that they were ionophores involved in the sequestration of iron from the soil, cf. [21], or in internal translocation. Detailed studies of the changes in the electronic spectra (250–700 nm) on mixing the pigments with various metal chlorides revealed very extensive chelation to Fe(II), much less to Fe(III) and Cu(II) (resulting in a nondescript grey-blue coloration), and no detectable complexing with Co(II), Zn(II), Ca(II), Mn(II) and Mg(II). However the ability for chelation of Fe(II) was little superior to that of the ubiquitously occurring caffeic acid when allowance was made for the dihydroxyphenyl units of the pigments acting as bidentate ligands. The lack of specificity for a unique valence state of a particular metal also probably rules out function as a true ionophore.

Studies were carried out to ascertain any toxicity of the pigments towards *Cladosporium herbarium*, a fungus which is a widespread plant pathogen. Colony formation was inhibited by 1 μg of the pigments, as shown by a simple plating assay (see Experimental). If the pigments are natural fungicides, their occurrence in healthy foliage could require their classification as inhibitions, i.e. compounds whose levels are boosted on infection [22]. In this context, the increased levels (cf. Table 1) found in tissue cultures could reflect enhancement triggered by the arguably stressed conditions, whereas the enhanced yields induced by increase of sucrose in the culture medium (Table 2) could result from the known properties of the sugar as an elicitor of phytoalexins *in vivo* [23].

EXPERIMENTAL

Culture methods. Specimens of *L. angustifolia* were obtained from the Royal Botanic Gardens, Kew; and from the Norfolk Lavender Farm, Heacham, Norfolk, U.K. All other plants and seeds were provided by Kew. Owing to the ready tendency to hydridize and the resulting difficulty in procuring pure *Metha* lines, certified specimens of *M. spicata* var. *lacinata* and *M. longifolia* L. (Kew catalogue numbers 240-54-2400 and 082-78-08852) were employed. Callus lines of *L. angustifolia* had been established over six years [1] and new lines were also developed from stem explants and cultured under slightly different conditions (either type of lines gave similar yields of pigments). The latter lines were initiated on Murashige and Skoog's medium [24] (MS: ex. Flow Labs., Irvine, Lanarkshire, U.K.) with the addition of either 2,4-D (6 mg/l) or NAA (0.5 mg/l) together with kinetin (0.1 mg/l), myoinositol (5 g/l), sucrose (20 g/l) and coconut milk (10% v/v): the medium was adjusted to pH 5.8 with aq. NaOH and supported on agar (oxoid No 3; 1.2% w/v). Cultures were maintained under two regimes: (a) subdued natural illumination (max. *ca* 400 lux) at 25° and (b): a diurnal variation of 12 hr illumination (Philips 'Growmore' fluorescent tubes; λ_{max} 660 nm; *ca* 2000 lux) at 30° followed by 12 hr dark at 15°. Explants (*ca* 10%) formed callus within 4 months and the passage time thereafter was 21 to 28 days. Callus lines under both regimes were white and friable with small localized blue-black areas; but blue pigmentation of the medium became noticeable after 2 to 3 days of sub-culture and such coloration was especially intense if Fe(III) was eliminated from the recipe for the culture medium and was replaced by $FeSO_4 \cdot 7H_2O$ (28 mg/l) and $Na_2EDTA \cdot 2H_2O$ (41 mg/l): ['NaFeEDTA' in commercially supplied media contains Fe(III)]. Suspension cultures were established on the MS basic medium with the same additives except agar. Inocula (2 to 4 g per 100 ml) yielded fine cell suspensions (*ca* 40% success) at 100 rpm under conditions (a), see above. The induction period was now *ca* 8 to 10 weeks, and subculture (12 ml inocula per 100 ml medium) was now made at *ca* 28 day intervals when the density was *ca* 1×10^6 cells/ml. Two-phase cultures utilized a layer (1 cm) of the triglyceride Miglyol 812 Neutralöl (ex. Dynamit Nobel (UK) Ltd.; Grangemouth) and immobilized cultures were prepared in polyurethane foam or gel matrix-films by standard methods [25].

Callus and suspension cultures of the other species were established from explants of stem from freshly germinated seeds. The former material was handled as described for *L. angustifolia* [1]: the seeds were sterilized with EtOH (30 sec) and $Ca(OCl)_2$ (1% aq.; 15–25 min), well-washed and layered on White's basal medium [26] in agar (1.2% w/v) at pH 5.8. After *ca* 15 days when shoot and root were *ca* 4 and 2 cm respectively, an explant (1 cm) of the hypocotyl region was initiated on M and S medium as above. Culture lines were all maintained on a 16–8 hr light-dark period with illumination and temperature as in regime (b). Typically, callus was induced within 6 to 8 weeks and the passage times were 3 to 4 weeks. All cultures were assayed for pigment after at least 6 and usually 10 passages. The new lines of *L. angustifolia* and those of *M. spicata* were maintained for up to $2\frac{1}{2}$ years (*ca* 20 passages) without apparent change in morphology or in biosynthetic capabilities. Suspensions of the latter species (passage time 4 to 6 weeks) were set up as for those of *L. angustifolia*.

Isolation of pigments. The enol esters were extracted from cell cultures with EtOAc and separated by TLC and HPLC as previously described [1]. For field-grown plants, the tissue (80 g) was frozen in liq. N_2 and pulverised and the resulting powder was extracted with Et_2O (300 ml) at 25° for 1 hr in the dark. The solvent was then decanted and evapd to dryness, and the residue partitioned between n-C_6H_{14}–MePh (1:1; 100 ml) and EtOH–H_2O (1:1; 100 ml). The non-aq. layer was then re-extracted with EtOH–H_2O (1:1; 100 ml; $\times 2$), following which the combined aq. fractions were extracted with n-C_6H_{14} (50 ml), and then reduced to half vol. and washed with EtOAc (100 ml; $\times 3$) after the addition of NaCl to control emulsions. The combined EtOAc extracts were then washed with aq. NaCl (10%; 100 ml), dried (Na_2SO_4) and evapd to dryness. The residue was dissolved in min. CH_2Cl_2 (*ca* 0.5 ml), silica gel H (0.25 g) added and after removal of the solvent the gel was layered on a column (10×1 cm) of the same material and the pigments (when present) were separated with an elution gradient of CH_2Cl_2–MeOH (0 to 10%, MeOH). The yellow fraction was isolated and purified by prep. TLC on silica gel PK6F (ex. Merck, Darmstadt, F.D.R.; 20 $\times$ 20 cm $\times$ 1000 μm) with MeOH–$CHCl_3$ (1:1) followed by recovery with MeOH. On analytical TLC (silica gel PK6F) the unresolved mixture of isomeric pigments had R_f values (solvent): 0.00 (MePH); 0.05 (EtOAc–$CHCl_3$ 1:3); 0.10 (Me_2CO–$CHCl_3$ 1:2); 0.33 (MeOH–$CHCl_3$ 3:17); and 0.39 (MeOH–CH_2Cl_2 3:17). The ratio of isomers was determined by ^{1}H NMR spectroscopy at 400 MHz [signals at δ5.67 and 6.36 respectively for the (*Z,E*) and (*E,E*)-compounds]. This pigments decomposed (*ca* 50%) within 6 months at 4° to form caffeic acid which was detected by ^{1}H NMR: presumably the putative dopaldehyde also generated underwent polymerization and yielded broad and hence undetected signals. The physical properties of the pigments have been recorded previously [1] but there the ^{1}H NMR

spectrum (at 200 MHz) was not fully resolved or analysed. We confirmed the structure of the (*Z,E*)-isomer and all the peaks were assigned by use of the COSY technique at 400 MHz which permits assignments and identification of hydrogens that share couplings. Contour plots allowed assignment of all ten atoms: (H-X is the hydrogen linked to C-X; carbons are numbered from the dopaldehyde moiety) δ[TMS = O; 5% soln in $(CD_3)_2CO$]: 5.67 (*d*, $J = 7.3$ Hz, H-7), 6.50 (*d*, $J = 15.9$ Hz, H-10), 6.81 (*d*, $J = 8.2$ Hz, H-5), 6.90 (*d*, $J = 8.2$ Hz, H-14), 7.00 (*dd*, $J = 8.2$, 2.1 Hz, H-6), 7.13 (*dd*, $J = 8.2$, 2.1 Hz, H-3), 7.25 (*d*, $J = 2$ Hz, H-17), 7.26 (*d*, $J = 7.3$ Hz, H-8), 7.36 (*d*, $J = 2.1$ Hz, H-2), 7.73 (*d*, $J = 15.9$ Hz, H-11).

Extraction of rosmarinic acid. Plant material (*ca* 40 g) was frozen in liq. N_2, pulverized, and extracted (Soxhlet) with EtOH–H_2O (7:3; 300 ml) for 24 hr. The liquid phase was concentrated (to half vol.), diluted with H_2O (50 ml) and extracted with EtOAc (100 ml; × 3). The combined EtOAc extracts were washed with satd aq. NaCl, and dried (Na_2SO_4). The aq. fractions were concd (to *ca* 1/3 vol.) and continuously extracted with EtOAc (100 ml) for 6 hr. The combined EtOAc fractions were then concd and subjected to prep. TLC on silica gel H (plate dimensions as before) with AcOH–MeOH–CH_2Cl_2 (4:15:35) as eluant and bands were visualised by spraying with phosphomolybdic acid (5% in EtOH) followed by heating (120°; 2 min). Under these conditions the pigments and rosmarinic acid had R_f values 0.88 and 0.70 respectively. Qualitative analyses (ca 1 μg detected) alone were carried out.

Isolation of phytosterol fraction of cultures. These compounds, together with any lower terpenoids if present, could be detected in the EtOAc extracts using capillary-GC [Pye Unicam 204 chromatograph; FI detector; OV 101 (≡SE30) 12 m × 0.32 mm id. W.C.O.T; 60° then gradient of 8°/min to 300° (20 min hold); 0.6 l N_2/hr: 1 μl injection (soln. 10% in Me_2CO) on column], and also by GC-MS using the same column coupled to a Kratos M25 mass spectrometer (ionising potential 70 eV) connected to a Kratos 65-505 data system. No lower terpenoids ($< 10^{-6}$% wt/fresh wt; if any) were detected, but sitosterol and stigmasterol were found at levels *ca* 10^{-3}% in cultures of *M. spicata*.

Callus of *L. angustifolia* was also extracted as follows. The initial EtOAc fraction (150 mg) extracted as for the assay of the pigments was suspended in *n*-C_6H_{14} (40 ml; 18 hr) at 20° and the supernatant was evapd to yield an oily solid (*ca* 30 mg). This was chromatographed on a column of silica gel with EtOAc–*n*-C_6H_{14} (gradient 0–16% EtOAc) which located the unresolved sterols in a fraction which on analytical-TLC on silica gel H with *n*-C_6H_{14}–EtOAc–$CHCl_3$ (4:1:1) had R_f 0.21. This mixture (11 mg) was subjected to HPLC on silica (5 μm grade; 4 columns, 10 × 0.45, 25 × 0.45, 25 × 0.45, 25 × 0.7 cm) with EtOAc–petrol (60–80°) (1:3) with a refractive index detector. After 6 cycles, the mixture was resolved into sitosterol and isofucosterol.

Fungotoxicity of pigments. Aliquots containing 1–100 μg of the pigments in EtOH were spotted onto a TLC plate (silica gel) which was then sprayed with an aq. nutrient soln containing spores of *C. herbarium*. The plate was enclosed in a plastic bag and incubated at 30° for several days. After this period the plate had developed a grey film of fungus and any inhibitory compounds were readily revealed as white spots. We thank Dr R. N. Strange (Deparment of Botany and Microbiology, UCL) for help with this test.

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REFERENCES

1. Banthorpe, D. V., Bilyard, H. J. and Watson, D. G. (1985) *Phytochemistry* **24**, 2677.
2. Arihara, S., Rüedi, P. and Eugster, C. H. (1975) *Helv. Chim. Acta* **58**, 447.
3. Lian-niang, L., Rui, T. and Wei-ming, C. (1984) *Planta Med.* **50**, 227.
4. Beiderbeck, R. (1982) *Z. Pflanzenphysiol.* **108**, 27.
5. Berlin, J., Witte, L., Schubert, W. and Wray, V. (1984) *Phytochemistry* **23**, 1277.
6. Charlwood, B. V. and Charlwood, K. A. (1983) *Biochem. Soc. Trans.* 592.
7. Allison, A. J., Butcher, D. N., Connolly, J. D. and Overton, K. H. (1968) *J. Chem. Soc., Chem. Commun.* 1493.
8. Hebbe, M. R., Narayanaswami, S. and Chadha, M. S. (1971) *Z. Naturforsch.* **26B**, 12.
9. Petit-Paly, G., Montagu, M., Viel, C., Rideau, M. and Chenieux, J-C. (1987) *Plant Cell Rep.* **6**, 809.
10. Withers, L. A. (1980) *Adv. Biochem. Eng.* **18**, 106.
11. Nakajima, N., Sonomoto, K., Usui, N., Sato, F., Yamada, Y., Tanaka, A. and Fukui, S. (1985) *J. Biotechnol.* **2**, 107.
12. Zenk, M. H., El-Shagi, H. and Ulbrich, B. (1977) *Naturwissenschafen* **64**, 585.
13. Harborne, J. B. (1966) *Z. Naturforsch.* **21B**, 604.
14. von Litvinenko, V. I., Popova, T. P., Simonjan, A. V., Zoz, T. G. and Sokolov, V. S. (1975) *Planta Med.* **27**, 373.
15. Tutin, T. G. *et al.* (1972) *Flora Europeae* Vol. 3, p. 126. Cambridge University Press, Cambridge.
16. Hegnauer, R. (1966) *Chemotaxonomie der Pflanzen* Vol. 4, p. 289. Birkhauser, Bäsel.
17. Angler, A. (1964) *Syllabus der Pflanzen Familien* (Melchoir, H., ed.). Vol. 2, p. 438. Borntraeger, Berlin.
18. Fodor, G. B. (1980) *Secondary Plant Products*, (Encyclopaedia of Plant Physiology, New Series, Vol. 8 (Bell, E. A. and Charlwood, B. V., eds), p. 100. Springer, Berlin.
19. Stökigt, J. and Zenk, M. H. (1974) *FEBS Letters* **42**, 131.
20. Watanabe, K., Sato, F., Furuta, M. and Yamada, Y. (1985) *Agric. Biol. Chem.* **49**, 533.
21. Chaney, R. L., Brown, J. C. and Tiffin, L. O. (1972) *Plant Physiol.* **50**, 208.
22. Ingham, J. L. (1973) *Phytopath. Z.* **78**, 314.
23. Cooksey, C. J., Garratt, P. J., Dahiya, J. S. and Strange, R. N. (1983) *Science* **220**, 1398.
24. Murashige, T. and Skoog, F. (1962) *Physiol. Plant* **15**, 473.
25. Rhodes, M. J. C., Smith, J. I. and Robins, R. J. (1987) *Appl. Microbiol. Biotechnol.* **26**, 28.
26. White, P. R. (1963) *Cultivation of Animal and Plant Cells*, 2nd Edn, p. 246. Ronald, New York.