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Neutral, alkaline and difference ultraviolet spectra of secondary metabolites from *Penicillium* and other fungi, and comparisons to published maxima from gradient high-performance liquid chromatography with diode-array detection

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ABSTRACT

The ultraviolet spectra of 6 predominantly secondary metabolites from filamentous fungi which, *inter alia*, are useful in the identification of the compounds after chromatography, were obtained in neutral (methanol) and alkaline solvents. Difference spectra were obtained by subtracting the neutral from the alkaline spectrum for each metabolite, using the spectrophotometer software. The data and method are of use in differentiating metabolites with similar chromophores. A database of the maxima was stored on a microcomputer for flexible storage, retrieval and updating of information. These data are compared to those published previously, obtained by diode-array detection using gradient high-performance liquid chromatography, which indicated that changes in solvent concentrations of the gradient affect the spectra of some metabolites. This could cause misidentification of chemosyndromes and metabolites which have been claimed to be of use in fungal chemotaxonomy.

INTRODUCTION

Fungal secondary metabolites are important because of the wide range of biological activities which the compounds can elicit. The effects can be beneficial (*e.g.* antibiotics) or detrimental (*e.g.* mycotoxins). Additional methods are required in the search to find new drugs, insecticides[1,2], herbicides, etc. derived from fungi, and to detect both established and potential mycotoxins. A concomitant need is to refine the species concepts in some groups of fungi to ensure that published work can be repeated

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with similar strains, and biochemical characters can contribute to this[3]. Towards this end, secondary metabolites have been increasingly emphasised as additional characters in fungal systematics[4].

Chromatography is often used to separate, and UV spectroscopy to identify, fungal secondary metabolites[5]. Spectra can be obtained from metabolites removed from (a) thin-layer chromatography (TLC) or preparative layer chromatography (PLC) plates[1] or (b) fractions from preparative high-performance liquid chromatography (HPLC); both these procedures permit additional analysis of the compounds to be made. Diode-array detection (DAD) with HPLC[6,7], and the recently developed technology to take UV spectra of spots on TLC plates[8] for the identification of fungal compounds, increasingly establishes the link between UV spectroscopy and chromatography.

The utility of UV spectroscopy can be increased for certain classes of compounds by comparing spectra of metabolites in neutral to those in strongly alkaline solvents, and subtracting the neutral from the alkaline spectrum to obtain a difference spectrum[9,10]. Difference curves are characteristic only of the ionizable elements of compounds and can be used to give quantitative and qualitative information on the compounds in mixtures. Also, the data and method are useful for the differentiation of metabolites with similar chromophores. Advantages are provided over absorption curves for the analyses of ionizable chromophores when the absorbing units are present either as parts of the molecule or in mixtures of various molecules. The method was primarily developed for the determination of phenolic components in lignin, but other ionizable chromophores which are ammenable to this technique are unsaturated systems containing, for example, enolic, carboxcylic and amino groups.

Difference curves permit the study of separate chromophores in complex mixtures without the need for physical separation and will be useful for the rapid determination of distinctive compounds in crude extracts of fungi. The technique will be useful in the identification of both fungi and compounds which the fungus has produced. Also, the UV spectra of certain metabolites which have similar UV spectra in neutral solvents can sometimes be differentiated by the UV spectra in alkaline solvents and/or the difference spectra.

A wide range of fungal metabolites has been used as taxonomic characters in *Penicillium*[3], and rapid and convenient methods are required to identify the metabolites. Frisvad and Filtenborg[4] published a list of TLC characteristics of selected secondary metabolites useful for systematic purposes in that genus. A standardized TLC database has also been made available on 80 identified and 27 unidentified fungal metabolites[11], and HPLC data bases have also been published[12]. Gradient HPLC–DAD of 182 secondary metabolites was published by Frisvad and Thrane[6], and the method was used to identify biosynthetically related metabolites on the basis of similar chromophores, which was claimed to be useful in the chemotaxonomy of fungi. References 6 and 12 are particularly useful in the identification of peaks obtained from HPLC of fungal extracts in other laboratories[2].

This paper provides the neutral, alkaline and difference spectra of 6 metabolites to assist in the identification of secondary metabolites with biological activity and those useful in taxonomy. These data are compared with the maxima published for the same metabolites obtained from gradient HPLC–DAD[6], to assess the validity of the methods used in refs. 6 and 7 for the identification of chromophores and metabolites of use in fungal systematics.

EXPERIMENTAL

Metabolites and purity

These were obtained from a collection of pure fungal metabolites held at CAB International Mycological Institute (Kew, U.K.). Purity of the metabolites was established before analysis as detailed in ref. 11, and 48 of the metabolites were also re-analysed for purity after UV analysis by both TLC systems. Six metabolites were analysed by HPLC[2] and assessed as pure, except for xanthocillin which has four peaks (also seen in ref. 6) which probably represent the methyl and dimethyl ether derivatives of xanthocillin; the spectral data are included in Tables I and II. UV spectra in methanol of some of the metabolites considered here have been published[5], and the spectra were compared to the maxima in ref. 6 (Table III).

UV spectra

All UV spectra were determined using a Philips PU8700 Series UV–VIS spectrophotometer. Spectra in the neutral solvent were obtained in HPLC-grade methanol; alkaline spectra were obtained by adding two drops of 2 *M* sodium hydroxide solution to the cuvette using a Pasteur pipette[13]. Difference spectra were obtained by using the graphic system of the spectrophotometer to subtract the neutral from the alkaline spectra. Molar extinction coefficients (ε) were not calculated because the metabolites were not weighed with a sufficiently sensitive weighing balance.

RESULTS AND DISCUSSION

Fig. 1 gives neutral, alkaline and difference spectra of some representative so-called acid, neutral and alkaloid metabolites with either two, three, four or five maxima in methanol. Table I provides the wavelength maxima of the neutral, alkaline and difference spectra for all metabolites. The spectra of the alkaloids cyclopenin and cyclopenol provide an example of the utility of the difference curves in differentiating between compounds with similar chromophores. Cyclopenin is converted into cyclopenol by cyclopenin *m*-hydroxylase which catalyses the addition of a hydroxyl group onto the phenyl ring. The UV maximum of cyclopenin is 289 nm, whereas that for cyclopenol is 282 nm, and both spectra are similar. However, the difference spectra of the two compounds are clearly different and can be used to differentiate between these similar compounds more effectively. Another example is that of the related compounds 3,5-dimethyl-6-methoxyphthalide and 3,5-dimethyl-6-hydroxyphthalide where the difference spectra are more distinct than the UV spectra in methanol. Other examples can be observed readily in Table II which lists the metabolites in order of increasing wavelength of the maxima, thereby listing together those metabolites with similar chromophores and enabling the rapid identification of spectra from unknown metabolites. Also, in some cases peaks were obtained with the alkaline and/or difference spectra where only end absorption was observed in methanol (*i.e.* hadacidin, canadensolide and β-nitropropionic acid), thus providing more information which can be used to identify these metabolites.

The UV maxima of the metabolites in methanol published here are compared to those published by Frisvad and Thrane[6], in an acetonitrile (with trifluoroacetic acid) and water solvent gradient, and to the methanol spectra of Cole and Cox[5] (Table III).

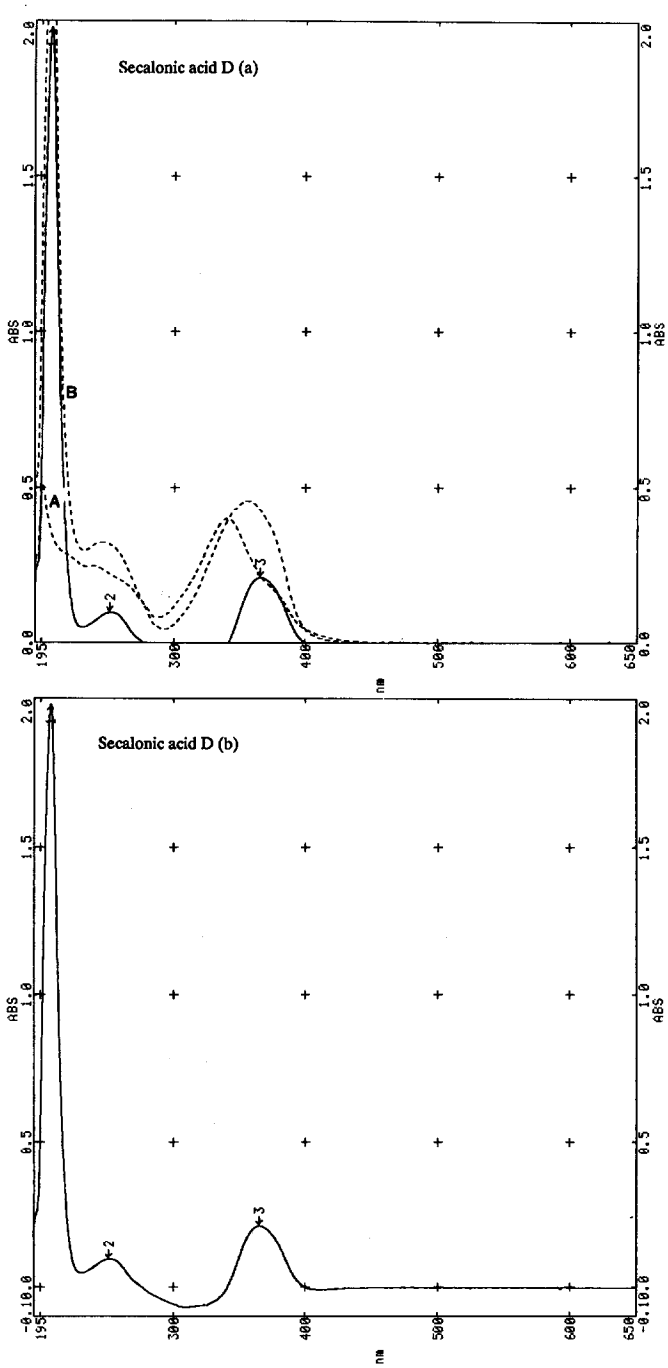

Fig. 1.

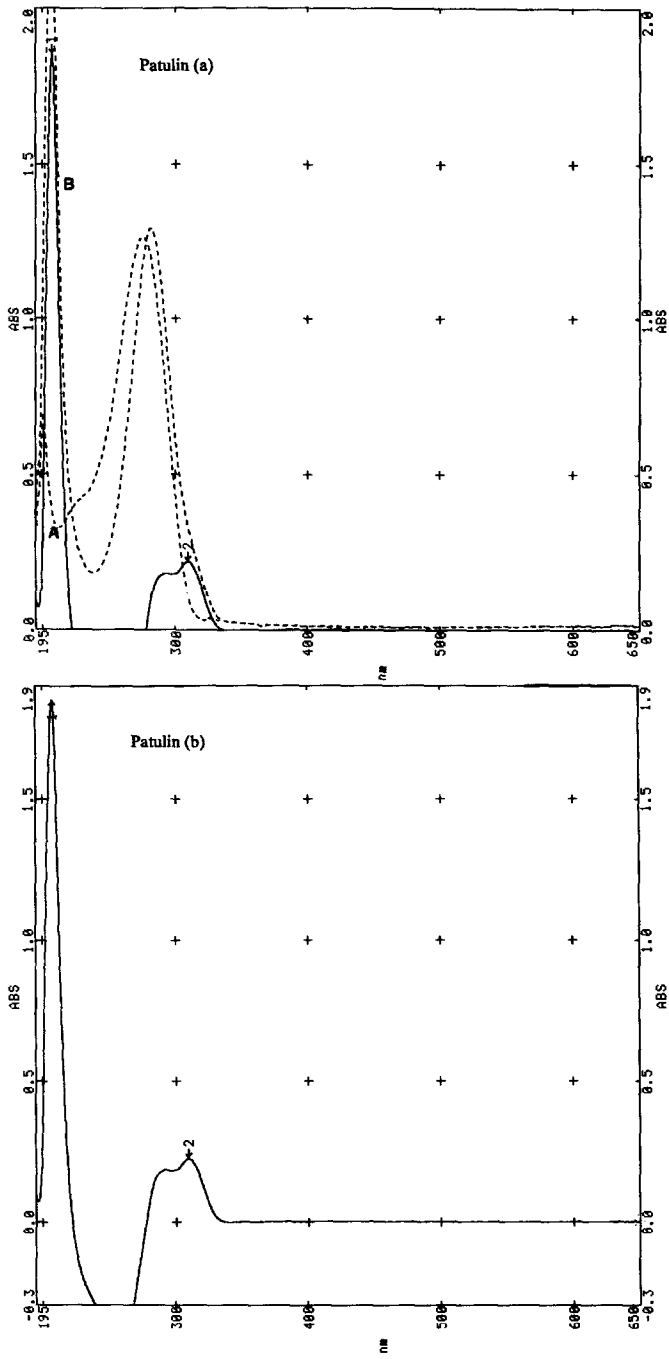

Fig. 1.

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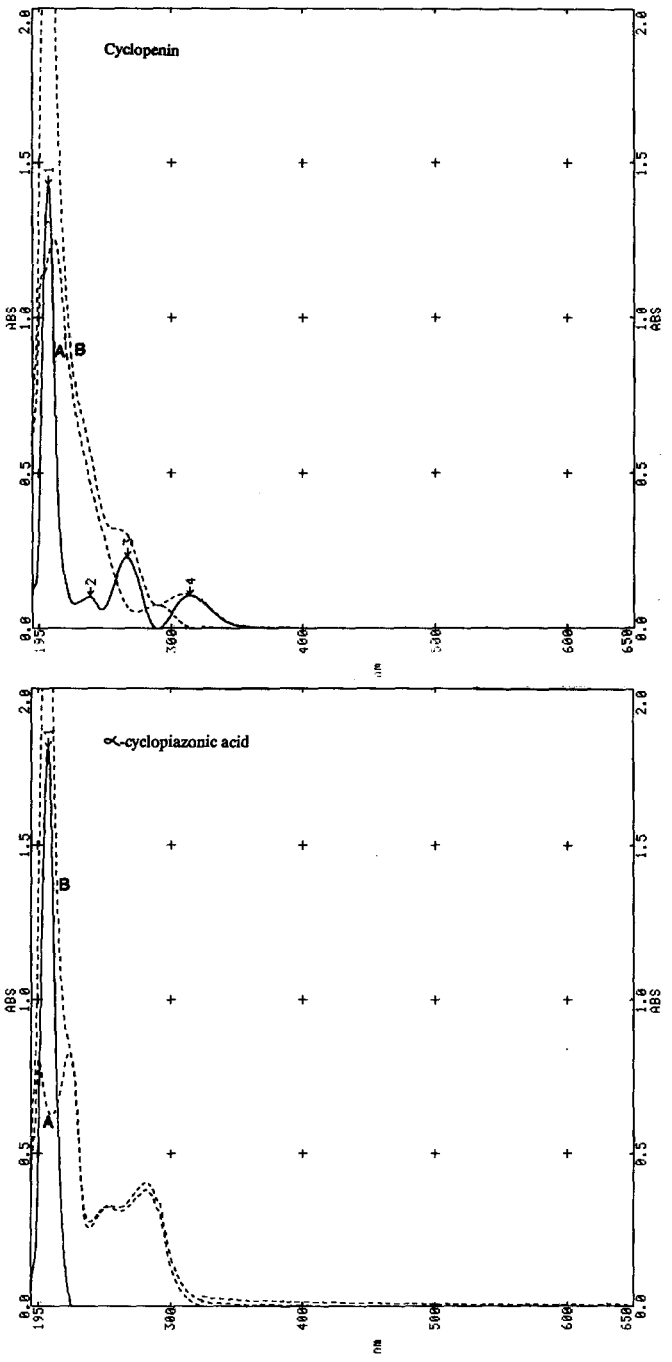

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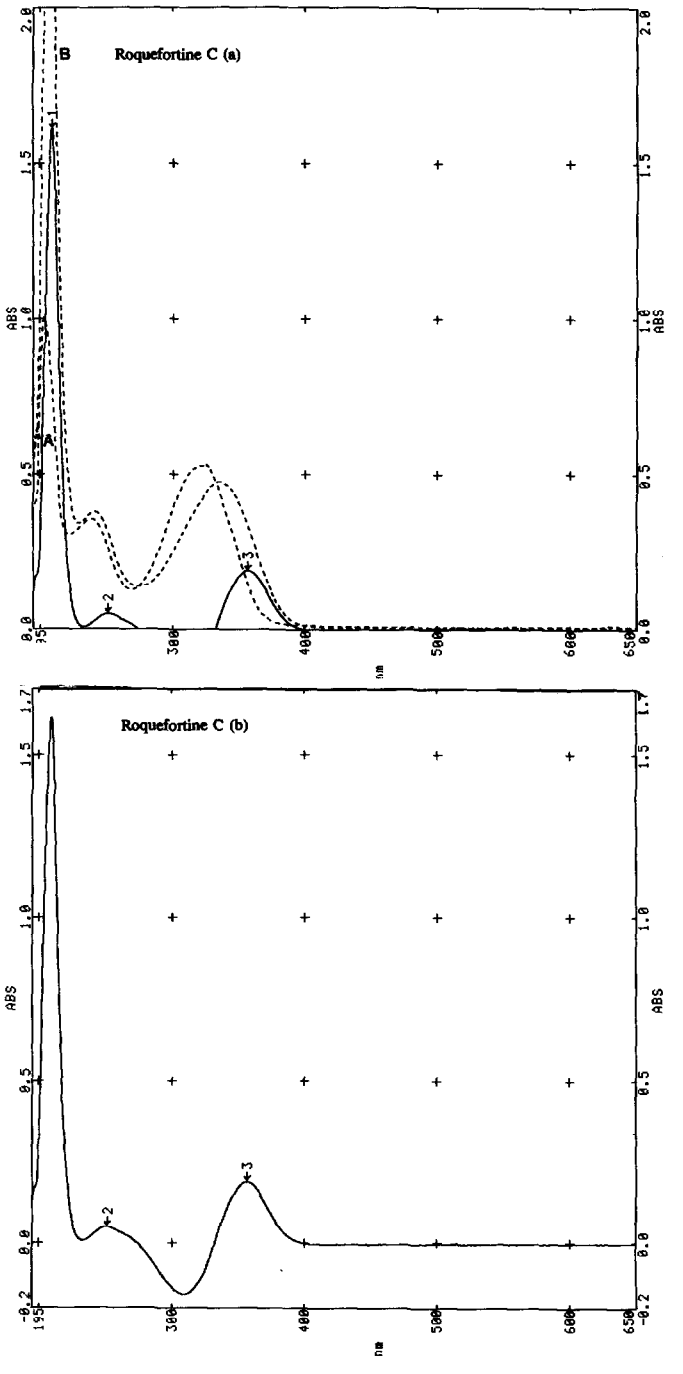

Fig. 1.

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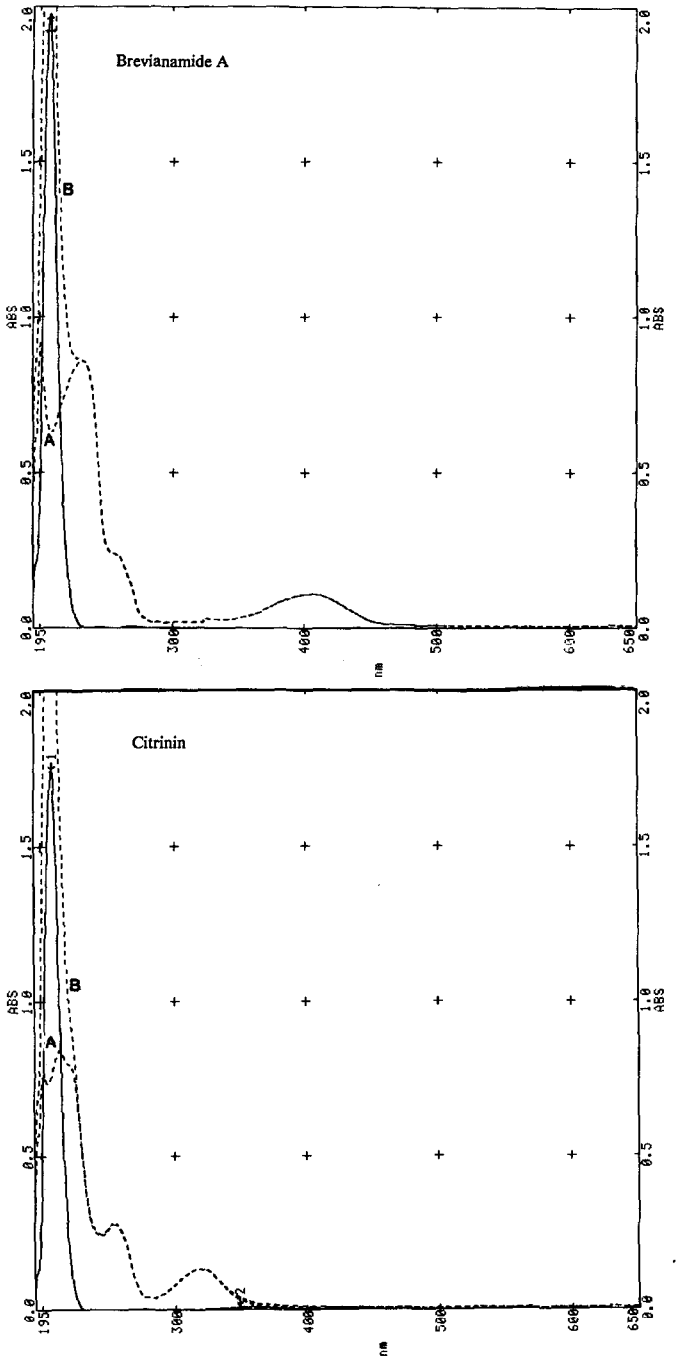

Fig. 1.

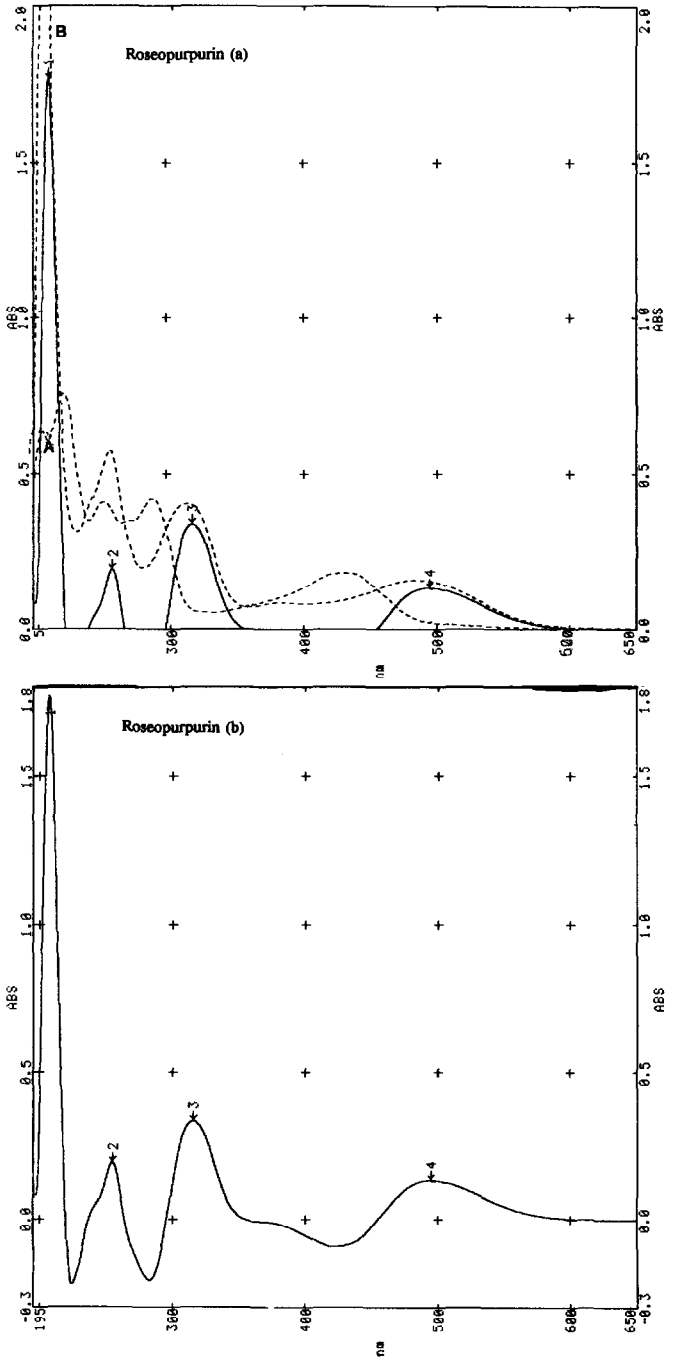

Fig. 1.

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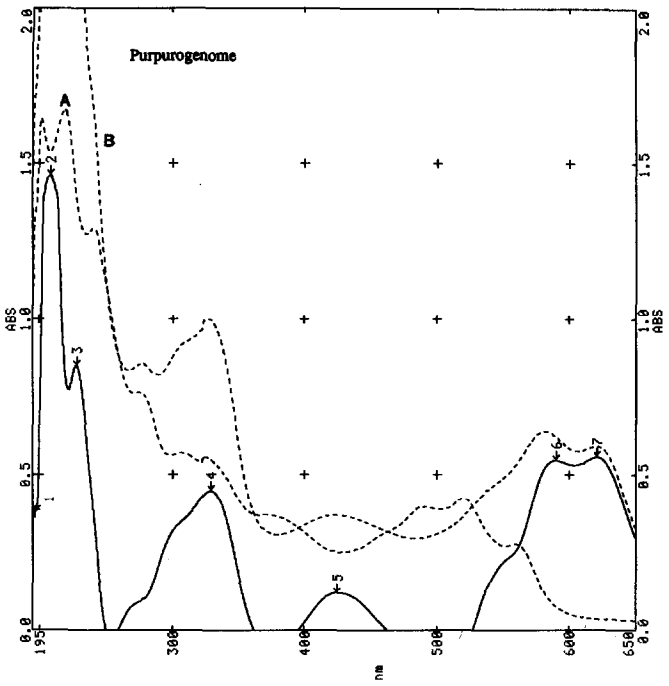

Fig. 1. Spectra of selected metabolites. Metabolites with one maximum are secalonic acid D (acid), patulin (neutral) and cyclopenin (alkaloid); two maxima are α-cyclopiazonic acid (acid), roquefortine C (neutral) and brevianamide A (alkaloid); three maxima is citrinin (acid); four maxima is roseopurpurin; five maxima is purpurogenome. (a) UV spectrum in methanol (dashed line A), plus sodium hydroxide (dashed line B) and difference spectrum (solid line), (b) = Difference spectrum alone.

An estimate of the similarity in the wavelength maxima for each metabolite is provided in Table IV. Although there was similarity to most of the maxima given by Frisvad and Thrane[6], the following was observed: (a) what were considered maxima by Frisvad and Thrane[6] are sometimes only shoulders or small peaks in the data obtained here (*e.g.* canescin, dipicolonic acid, etc.; Table III); (b) some maxima reported in ref. 6 were not observed in the new data set, and *vice versa* (*e.g.* carolic acid, chaetoglobosin C, etc.; Table III); (c) maxima had different values for the same metabolites (*e.g.* 3,5-dimethyl-6-hydroxyphthalide, fulvic acid, etc.; Table III). This indicates considerable differences in the two data sets, and not slight differences as observed by Frisvad and Thrane[6] when comparing their data to those of Cole and Cox[5].

The similarity in the data appears to be correlated with the retention indices of the metabolites[6] and consequently the particular solvent composition in which they were dissolved at the time of elution. The largest discrepancy between the present results and those of Frisvad and Thrane[6] occur at retention indices (*I*) of between 1000 and 1299 (Table IV). The differences which occurred in this range are, for example: the chaetoglobosin C peak at 252 nm in ref. 6 was not observed in the present study or in ref. 5, and shoulders and small peaks were observed here rather than the maxima as given in ref. 6; the α-cyclopiazonic acid shoulder at 253 nm was observed in the present

TABLE I

MAXIMA (AS ASSESSED BY THE SPECTROPHOTOMETER COMPUTER) OF THE SECONDARY METABOLITES IN METHANOL, METHANOL PLUS TWO DROPS OF 2 *M* SODIUM HYDROXIDE AND DIFFERENCE SPECTRA

λ = Wavelength; *A* = absorbance.

Metabolite		*Methanol*		*Sodium hydroxide*		*Difference*	
		λ *(nm)*	*A*	λ *(nm)*	*A*	λ *(nm)*	*A*
Aurofusarin	(1)	201.1	0.413	207.3	2.168	207.5	1.931
(4.0 μg ml^{-1})	(2)	253.7	0.189	251.4	0.199		
Austdiol	(1)	201.7	0.761	208.1	2.276	208.5	1.763
(4.0 μg ml^{-1})	(2)	256.4	0.517	256.3	0.445	312.0	0.042
	(3)	380.0	0.853	380.0	0.756	407.6	0.037
Brefeldin A	(1)	203.6	1.584	209.1	2.518	209.6	1.107
(9.0 μg ml^{-1})							
Brevianamide A	(1)	200.9	0.917	207.8	2.607	207.9	1.977
(3.2 μg ml^{-1})	(2)	232.0	0.859	405.6	0.110		
	(3)	406.2	0.113				
Canadensic acid	(1)	202.9	0.903	208.4	2.138	208.9	1.475
(10.0 μg ml^{-1})							
Canadensolide	(1)	203.3	0.849	207.8	2.618	208.0	1.811
(6.6 μg ml^{-1})	(2)					244.1	0.527
Canescin	(1)	200.1	0.516	206.6	2.235	206.7	1.897
(1.6 μg ml^{-1})	(2)	247.3	1.053	257.9	0.920	244.0	−0.347
	(3)	327.4	0.147	284.0	0.329	258.5	0.536
	(4)			313.0	0.315	285.7	0.183
	(5)					309.0	0.212
Carlosic acid	(1)	199.8	0.551	206.7	2.439	206.7	2.220
(4.0 μg ml^{-1})	(2)	230.9	0.870	230.9	0.892	256.8	0.073
	(3)	265.8	0.963	264.6	1.002		
Carolic acid	(1)	196.5	0.432	206.0	2.273	206.0	1.783
(4.0 μg ml^{-1})	(2)	205.6	0.490	233.6	0.675	248.1	0.360
	(3)	230.2	0.574	256.0	0.883		
	(4)	269.1	0.972				
Chaetoglobosin C	(1)	202.2	1.347	209.9	2.989	209.9	1.961
(2.8 μg ml^{-1})	(2)	221.3	1.273				
Cinnamic acid	(1)	203.2	0.889	207.7	2.364	207.9	1.590
(1.8 μg ml^{-1})	(2)	215.2	0.773	267.2	0.819	242.0	0.082
	(3)	221.0	0.633				
	(4)	269.7	0.829				
Citrinin	(1)	201.3	0.764	208.5	2.514	208.2	1.742
(3.2 μg ml^{-1})	(2)	213.7	0.840	254.5	0.277		
	(3)	254.6	0.283	319.8	0.132		
	(4)	320.1	0.131				
Compactin	(1)	198.7	0.573	206.9	2.515	206.9	2.083
(6.6 μg ml^{-1})	(2)	229.5	1.154	229.5	1.149	240.6	0.006
	(3)	236.7	1.265	236.7	1.247	249.2	0.023
	(4)	245.1	0.836	245.1	0.832		

(Continued on p. 206)

TABLE I *(continued)*

Metabolite		*Methanol*		*Sodium hydroxide*		*Difference*	
		λ (nm)	*A*	*λ (nm)*	*A*	*λ (nm)*	*A*
Cyclopaldic acid	(1)	202.3	1.296	206.4	2.483	207.6	1.593
(5.0 μg ml^{-1})	(2)	243.9	1.080	393.3	0.076	298.3	0.071
	(3)					398.6	0.069
Cyclopenin	(1)	212.0	1.255	208.2	2.626	207.7	1.427
(4.8 μg ml^{-1})	(2)	288.8	0.076	312.8	0.111	239.3	0.102
	(3)					267.3	0.229
	(4)					314.5	0.106
Cyclopenol	(1)	203.1	1.405	208.8	2.592	209.5	1.620
(1.8 μg ml^{-1})	(2)	281.6	0.095			308.7	0.072
α-Cyclopiazonic acid	(1)	201.3	0.806	207.9	2.456	208.0	1.812
(3.2 μg ml^{-1})	(2)	224.5	0.828	253.6	0.327	250.8	−0.004
	(3)	281.6	0.407	281.8	0.382		
Cytochalasin H	(1)	202.9	1.058	208.9	2.322	209.5	1.479
(3.3 μg ml^{-1})							
Desacetylpebrolide	(1)	201.5	0.986	206.5	2.247	206.9	1.805
(11.9 μg ml^{-1})	(2)	229.6	0.794	229.6	0.786		
Dehydrocanadensolide	(1)	200.6	0.386	212.9	2.910	213.4	2.629
(40 μg ml^{-1})							
Dehydrocarolic acid	(1)	199.6	0.666	206.8	2.483	206.8	1.896
(9.0 μg ml^{-1})	(2)	244.1	0.806	247.1	1.024	248.1	0.225
	(3)	285.6	0.861	300.8	0.476	317.0	0.154
Desmethoxyviridiol	(1)	201.1	0.491	207.2	2.356	207.5	2.011
(1.6 μg ml^{-1})	(2)	248.2	0.530	232.8	0.484	292.8	0.655
	(3)	318.9	0.265	293.5	0.793	368.9	0.098
	(4)			368.9	0.107	459.5	0.198
	(5)			460.8	0.198		
3,5-Dimethyl-6-	(1)	208.7	1.299	207.3	2.411	207.1	1.126
-hydroxyphthalide	(2)	242.4	0.236	224.4	1.159	225.1	0.896
(1.6 μg ml^{-1})	(3)	312.8	0.151	247.2	0.240	253.5	0.163
	(4)			351.2	0.251	351.4	0.235
3,5-Dimethyl-6-	(1)	207.9	1.835	210.2	2.448	211.7	0.682
-methoxyphthalide	(2)	239.8	0.398	240.4	0.383		
(1.6 μg ml^{-1})	(3)	300.0	0.180	300.0	0.173		
Dipicolonic acid	(1)	202.6	1.319	206.5	2.177	207.6	1.300
(10.0 μg ml^{-1})	(2)	269.7	0.269	269.9	0.296	279.0	0.035
Duclauxin	(1)	201.3	0.898	207.1	2.672	194.4	0.261
(not determined)	(2)	232.1	0.872	240.5	0.788	207.3	1.880
	(3)			319.5	0.541	252.9	0.089
	(4)			415.6	0.170	322.0	0.358
	(5)					415.7	0.119
Epoxysuccinate	(1)	200.8	0.393	207.6	2.058	207.7	1.768
(40.0 μg ml^{-1})							
(29) Ergosterol	(1)	201.7	0.974	206.4	2.229	207.0	1.544
(10.0 μg ml^{-1})	(2)	271.9	0.615	271.8	0.621		
	(3)	282.1	0.638	282.1	0.644		
	(4)	293.8	0.379	293.8	0.388		

TABLE I *(continued)*

Metabolite		*Methanol*		*Sodium hydroxide*		*Difference*	
		λ (nm)	*A*	*λ (nm)*	*A*	*λ (nm)*	*A*
Ethisolide (3.3 μg ml^{-1})	(1)	201.3	0.689	208.0	2.216	208.2	1.712
Frequentin (3.3 μg ml^{-1})	(1)	198.9	0.769	207.3	2.693	207.1	2.122
	(2)	231.5	1.286	227.2	1.183	252.1	0.066
	(3)	288.8	0.213	312.8	0.636	314.1	0.510
Fulvic acid (3.3 μg ml^{-1})	(1)	200.7	0.631	207.8	2.340	207.9	1.882
	(2)	376.3	0.307	368.9	0.351	246.5	0.075
	(3)					266.8	0.072
	(4)					358.0	0.052
Gentisic acid (3.3 μg ml^{-1})	(1)	202.5	0.950	208.1	2.321	208.1	1.426
	(2)	327.6	0.170	330.4	0.144	252.5	0.053
	(3)					364.3	0.032
Gentisyl alcohol (4.0 μg ml^{-1})	(1)	202.7	1.164	207.7	2.350	208.5	1.760
	(2)	296.8	0.205	268.3	0.476	266.0	0.449
	(3)					321.0	0.058
Glauconic acid (4.0 μg ml^{-1})	(1)	203.2	0.877	207.7	2.283	207.9	1.462
Gliotoxin (8.0 μg ml^{-1})	(1)	202.6	1.016	208.5	2.408	209.1	1.666
	(2)	268.3	0.295	267.2	0.300		
Griseofulvin (16.0 μg ml^{-1})	(1)	196.5	0.642	208.4	2.156	206.6	1.688
	(2)	212.7	0.769	232.9	0.704	259.9	−0.002
	(3)	233.1	0.724	251.8	0.737	299.2	0.012
	(4)	251.7	0.744	288.1	1.361		
	(5)	287.9	1.376				
Griseophenone C (2.8 μg ml^{-1})	(1)	205.3	1.796	207.5	2.655	209.2	1.018
	(2)	296.1	0.683	302.4	0.611	246.2	0.471
	(3)			380.8	0.190	311.1	0.103
	(4)					389.9	0.164
Hadacidin (4.0 μg ml^{-1})	(1)	204.1	0.990	207.2	2.650	207.3	1.681
	(2)					237.5	0.747
5′-Hydroxyasperentin (4.8 μg ml^{-1})	(1)	201.1	0.740	208.1	2.373	207.7	1.587
	(2)	216.1	0.940	246.2	0.421	242.9	0.299
	(3)	268.8	0.577	310.8	1.114	313.2	0.933
	(4)	301.3	0.252				
m-Hydroxybenzoic acid (1.8 μg ml^{-1})	(1)	207.7	1.589	209.1	2.581	209.9	1.020
	(2)	297.3	0.146	312.0	0.120	218.5	0.900
	(3)					249.7	0.179
	(4)					320.7	0.091
p-Hydroxybenzoic acid (4.0 μg ml^{-1})	(1)	202.9	1.315	208.9	2.455	209.9	1.527
	(2)	252.2	0.899	276.0	1.098	286.1	0.954
Hydroxyisocanadensic acid (4.4 μg ml^{-1})	(1)	200.1	0.494	207.7	2.326	207.7	1.985
	(2)	225.2	0.452				
Itaconic acid (4.0 μg ml^{-1})	(1)	202.8	0.880	208.1	2.202	208.7	1.519

(Continued on p. 208)

TABLE I *(continued)*

Metabolite		*Methanol*		*Sodium hydroxide*		*Difference*	
		λ (nm)	*A*	*λ (nm)*	*A*	*λ (nm)*	*A*
Kojic acid	(1)	200.4	0.780	206.7	2.576	206.6	1.874
(4.0 μg ml^{-1})	(2)	217.5	0.906	226.9	1.405	229.2	0.841
	(3)	270.4	0.501	314.4	0.337	315.7	0.322
Lapidosin	(1)	199.8	0.575	207.4	2.449	207.4	1.970
(3.3 μg ml^{-1})	(2)	229.1	0.570	259.5	0.683	257.6	0.341
	(3)	320.2	0.551	305.4	0.619	303.4	0.103
	(4)					359.5	0.171
Lichexanthone	(1)	201.1	0.723	207.7	2.479	207.9	1.890
(4.0 μg ml^{-1})	(2)	241.7	0.621	240.0	0.586	271.3	0.081
	(3)	307.2	0.373	306.1	0.312	375.3	0.038
6-Methylsalicylic acid	(1)	209.7	0.969	209.3	2.344	209.1	1.376
(1.6 μg ml^{-1})	(2)	311.0	0.124	300.8	0.114	290.6	0.017
Monorden	(1)	201.9	1.062	207.0	2.541	207.3	1.561
(4.0 μg ml^{-1})	(2)	266.4	0.526	253.6	0.573	248.6	0.199
	(3)			275.0	0.633	277.7	0.134
	(4)			318.9	0.354	324.0	0.231
Mycelianamide	(1)	201.6	0.745	208.2	2.305	208.7	1.881
(4.0 μg ml^{-1})	(2)	231.2	0.239	328.9	0.398	276.6	0.017
	(3)	324.2	0.406			357.9	0.127
Mycophenolic acid	(1)	201.9	0.949	208.1	2.497	207.9	1.633
(3.2 μg ml^{-1})	(2)	215.8	0.997	345.3	0.176	229.4	0.480
	(3)	250.1	0.226			262.5	0.025
	(4)	305.9	0.115			345.3	0.151
β-Nitropropionic acid	(1)	201.5	0.645	207.1	2.340	207.5	1.880
(4.0 μg ml^{-1})	(2)			236.0	0.467	237.8	0.423
Norlichexanthone	(1)	201.0	0.840	207.3	2.513	207.5	1.867
(3.3 μg ml^{-1})	(2)	241.3	0.797	237.6	0.690	247.7	−0.025
	(3)	311.1	0.468	266.4	0.462	267.0	0.232
	(4)			360.5	0.728	362.9	0.653
Orsellenic acid	(1)	200.6	0.800	207.1	2.432	206.9	1.719
(1.6 μg ml^{-1})	(2)	217.5	1.017	275.6	0.477	236.8	0.237
	(3)	259.2	0.411			277.4	0.357
	(4)	300.0	0.176				
Palitantin	(1)	198.5	0.761	206.6	2.674	206.5	2.082
(4.9 μg ml^{-1})	(2)	232.0	1.430	231.7	1.396	251.0	0.012
Patulin	(1)	200.7	0.700	207.4	2.226	207.7	1.852
(3.2 μg ml^{-1})	(2)	275.7	1.263	281.4	1.293	309.4	0.221
Paxilline	(1)	199.2	0.568	206.8	2.523	206.8	2.039
(1.6 μg ml^{-1})	(2)	230.5	0.940	230.1	0.938		
	(3)	281.6	0.201	280.1	0.204		
Penicillic acid	(1)	200.7	0.656	208.7	2.583	208.5	2.051
(3.2 μg ml^{-1})	(2)	224.8	0.800			251.5	0.087
Penitrem A	(1)	200.9	0.701	206.7	2.356	206.8	1.771
(7.4 μg ml^{-1})	(2)	234.9	0.666	234.6	0.662		
	(3)	301.9	0.215	301.6	0.215		

TABLE I *(continued)*

Metabolite		*Methanol*		*Sodium hydroxide*		*Difference*	
		λ (nm)	*A*	*λ (nm)*	*A*	*λ (nm)*	*A*
Phoenicin	(1)	200.7	0.872	207.2	2.785	207.2	2.014
(4.0 μg ml^{-1})	(2)	217.6	0.829	269.1	0.666	287.0	0.093
	(3)	261.6	0.821	527.3	0.123	577.9	0.053
	(4)	492.8	0.105				
PR-Toxin	(1)	201.5	0.920	208.3	2.479	208.7	1.952
(4.6 μg ml^{-1})	(2)	247.5	0.653	248.3	0.621	402.7	0.027
	(3)			400.3	0.054		
Purpurogenone	(1)	201.9	1.647	203.5	3.008	195.9	0.381
(20.0 μg ml^{-1})	(2)	219.5	1.674	278.2	0.859	208.5	1.462
	(3)	241.4	1.291	327.3	1.001	228.3	0.851
	(4)			423.5	0.370	329.2	0.450
	(5)	519.5	0.424	581.6	0.639	424.9	0.119
	(6)			621.9	0.592	590.6	0.546
	(7)					621.9	0.557
2-Pyruvoylamino-	(1)	211.2	0.920	206.9	2.467	206.9	1.563
benzamide	(2)	248.0	0.460	309.9	0.278	236.8	0.064
(4.0 μg ml^{-1})	(3)	301.3	0.228			321.9	0.127
Roquefortine B	(1)	202.2	0.937	207.0	2.359	207.3	1.527
(4.0 μg ml^{-1})	(2)	223.7	1.234	281.1	0.272		
	(3)	281.9	0.272				
Roquefortine C	(1)	203.1	1.005	208.9	2.406	209.6	1.614
(4.8 μg ml^{-1})	(2)	238.6	0.355	241.8	0.380	251.6	0.050
	(3)	324.1	0.536	336.5	0.477	357.0	0.189
Roseopurpurin	(1)	201.0	0.648	207.8	2.396	207.7	1.773
(4.0 μg ml^{-1})	(2)	218.5	0.760	253.8	0.576	255.0	0.193
	(3)	248.3	0.407	311.7	0.402	315.6	0.336
	(4)	285.7	0.419	482.8	0.160	494.3	0.134
	(5)	429.6	0.183				
Rubratoxin B	(1)	202.3	0.956	208.2	2.319	208.7	1.539
(3.2 μg ml^{-1})							
Rugulovasine A	(1)	203.6	1.069	207.7	2.503	207.7	1.450
(4.0 μg ml^{-1})	(2)	221.2	1.183	287.0	0.231		
	(3)	287.2	0.229				
Secalonic acid D	(1)	201.1	0.511	207.9	2.329	208.1	1.980
(4.0 μg ml^{-1})	(2)	340.8	0.400	247.2	0.324	251.7	0.097
	(3)			356.0	0.459	364.9	0.211
Scytalone	(1)	200.7	0.704	207.1	2.280	207.0	1.717
(1.6 μg ml^{-1})	(2)	221.3	0.710	252.0	0.264	225.7	−0.061
	(3)	282.1	0.651	330.4	1.226	250.0	0.191
	(4)					332.1	1.039
Spinulosin	(1)	210.4	0.529	206.5	2.218	206.5	1.709
(1.6 μg ml^{-1})	(2)	301.1	0.317	330.5	0.612	332.5	0.466
Sterigmatocystin	(1)	201.9	0.736	206.9	2.568	207.1	1.871
(2.0 μg ml^{-1})	(2)	246.4	0.839	240.0	0.885	238.5	0.089
	(3)	326.5	0.398	324.0	0.355	274.7	0.041
	(4)					389.7	0.031

(Continued on p. 210)

TABLE I *(continued)*

Metabolite		*Methanol*		*Sodium hydroxide*		*Difference*	
		λ (nm)	*A*	*λ (nm)*	*A*	*λ (nm)*	*A*
Stipitatic acid	(1)	200.1	0.738	207.1	2.543	207.2	1.977
(1.6 μg ml^{-1})	(2)	263.0	1.602	264.4	1.454	282.8	0.456
	(3)	324.1	0.190	333.8	0.284	333.8	0.112
	(4)	369.1	0.237			404.8	0.017
Terrestric acid	(1)	201.3	0.569	206.1	2.288	206.3	1.764
(1.6 μg ml^{-1})	(2)	228.5	0.365	254.1	0.805	248.6	0.429
	(3)	272.6	0.924				
Viomellein	(1)	200.7	0.562	207.7	2.426	207.9	2.009
(4.0 μg ml^{-1})	(2)	222.4	0.448	264.5	0.483	316.2	0.019
	(3)	264.1	0.537	382.4	0.140	548.2	0.056
	(4)	380.3	0.154	528.5	0.073		
Viridicatum toxin	(1)	201.2	0.580	207.7	2.277	208.1	1.951
(not determined)	(2)	235.2	0.223	280.1	0.263		
	(3)	283.2	0.289	440.8	0.092		
	(4)	449.6	0.086				
Xanthocillin	(1)	201.1	0.792	208.1	2.524	208.5	2.009
(4.0 μg ml^{-1})	(2)	346.4	0.405	251.2	0.454	266.6	0.249
	(3)			338.4	0.443	335.2	0.065
	(4)			410.4	0.390	416.1	0.359

TABLE II

METABOLITES LISTED BY INCREASING WAVELENGTH (nm) OF MAXIMA

In lists 2–8 end absorption peaks have been omitted.

Methanol	*Sodium hydroxide*	*Difference*	*Metabolite*
(1) End absorption only			
200.6	212.9	213.4	Dehydrocanadensolide
200.8	207.6	207.7	Epoxysuccinate
201.3	208.0	208.2	Ethisolide
202.3	208.2	208.7	Rubratoxin B
202.8	208.1	208.7	Itaconic acid
202.9	208.4	208.9	Canadensic acid
202.9	208.9	209.5	Cytochalasin H
203.2	207.7	207.9	Glauconic acid
203.6	209.1	209.6	Brefeldin A
(2) Difference peak only			
		237.5	Hadacidin
		244.1	Canadensolide
(3) Sodium hydroxide and difference peak only			
	236.0	237.8	β-Nitropropionic acid

TABLE II *(continued)*

Methanol	*Sodium hydroxide*	*Difference*	*Metabolite*
(4) One maximum in methanol			
221.3	–	–	Chaetoglobosin C
224.8	–	251.5	Penicillic acid
225.2			Hydroxyisocanadensic acid
229.6	229.6		Desacetylpebrolide
232.0	231.7	251.0	Palitantin
232.1	240.5 319.5 415.6	207.3 252.9 322.0 415.7	Duclauxin
243.9	393.3	298.3 398.6	Cyclopaldic acid
247.5	248.3 400.3	272.1 402.7	PR-Toxin
252.2	276.0	286.1	*p*-Hydroxybenzoic acid
253.7	251.4		Aurofusarin
266.4	253.6 275.0 318.9	248.6 277.7 324.0	Monorden
268.3	267.2		Gliotoxin
269.7	269.9	279.0	Dipicolonic acid
275.7	281.4	309.4	Patulin
281.6		308.7	Cyclopenol
288.8	312.8	239.3 267.3 314.5	Cyclopenin
296.1	302.4 380.8	246.2 311.1 389.9	Griseophenone C
296.8	268.3	266.0 321.0	Gentisyl alcohol
297.3	312.0	218.5 249.7 320.7	*m*-Hydroxybenzoic acid
301.1	330.5	332.5	Spinulosin
311.0	300.8	290.6	6-Methylsalicylic acid
327.6	330.4	252.5 364.3	Gentisic acid
340.8	247.2 356.0	251.7 364.9	Secalonic acid D
346.4	251.2 338.4 410.4	266.6 335.2 416.1	Xanthocillin

(Continued on p. 212)

TABLE II *(continued)*

Methanol	*Sodium hydroxide*	*Difference*	*Metabolite*
376.3	368.9	246.5 266.8 358.0	Fulvic acid
(5) Two maxima in methanol			
217.5 270.4	226.9 314.4	229.2 315.7	Kojic acid
221.3 282.3	252.0 330.4	225.7 250.0 332.1	Scytalone
221.2 287.2	287.0		Rugulovasine A
223.7 281.9	281.1		Roquefortine B
224.5 281.6	253.6 281.8	250.8	α-Cyclopiazonic acid
228.5 272.6	254.1	248.6	Terrestric acid
229.1 320.2	259.5 305.4	257.6 303.4 359.5	Lapidosin
230.9 265.8	230.9 264.6	256.8	Carlosic acid
230.5 281.6	230.1 280.1		Paxilline
231.5 288.8	227.2 312.8	252.1 314.1	Frequentin
231.2 324.2	328.9	276.6 357.9	Mycelianamide
232.0 406.2	405.6		Brevianamide A
234.9 301.9	234.6 301.6		Penitrem A
238.6 324.1	241.8 336.5	251.6 357.0	Roquefortine C
239.8 300.0	240.4 300.0		3,5-Dimethyl-6-methoxyphthalide
241.3 311.1	237.6 266.4 360.5	247.7 267.0 362.9	Norlichexanthone
241.7 307.2	240.0 306.1	271.3 375.3	Lichexanthone
242.4 312.8	224.4 247.2 351.2	225.1 253.5 351.4	3,5-Dimethyl-6-hydroxyphthalide

TABLE II *(continued)*

Methanol	*Sodium hydroxide*	*Difference*	*Metabolite*
244.1	247.1	248.1	Dehydrocarolic acid
285.6	300.8	317.0	
246.4	240.0	238.5	Sterigmatocystin
326.5	324.0	274.7	
		389.7	
247.3	257.9	244.0	Canescin
327.4	284.0	258.5	
	313.0	285.7	
		309.0	
248.2	309.9	236.8	2-Pyruvoylaminobenzamide
301.3		321.9	
248.2	232.8	292.8	Desmethoxyviridiol
318.9	293.5	368.9	
	368.9	459.5	
	460.8		
256.4	256.3	312.0	Austdiol
380.0	380.0	407.6	
(6) Three maxima in methanol			
205.6	233.6	248.1	Carolic acid
230.2	256.0		
269.1			
213.7	254.5		Citrinin
254.6	319.8		
320.1			
215.2	267.2	242.0	Cinnamic acid
221.0			
269.7			
215.8	345.3	229.4	Mycophenolic acid
250.1		262.5	
305.9		345.3	
216.1	246.2	242.9	5′-Hydroxyasperentin
268.8	310.8	313.2	
301.3			
217.5	275.6	236.8	Orsellenic acid
259.2		277.4	
300.0			
217.6	269.1	287.0	Phoenicin
261.6	527.3	577.9	
492.8			
222.4	264.5	316.2	Viomellein
264.1	382.4	548.2	
380.3	528.5		
229.5	229.5	240.6	Compactin
236.7	236.7	249.2	
245.1	245.1		

(Continued on p. 214)

TABLE II *(continued)*

Methanol	*Sodium hydroxide*	*Difference*	*Metabolite*
235.2	280.1		Viridicatum toxin
283.2	440.8		
449.6			
263.0	264.4	282.8	Stipitatic acid
324.1	333.8	333.8	
369.1		404.8	
271.9	271.8	258.7	Ergosterol
282.1	282.1	268.1	
293.8	293.8	279.3	
		291.6	
(7) Four maxima in methanol			
212.7	232.9	259.9	Griseofulvin
233.1	251.8	299.2	
251.7	288.1		
287.9			
218.5	253.8	255.0	Roseopurpurin
248.3	311.7	315.6	
285.7	482.8	494.3	
429.6			
(8) Five maxima in methanol			
219.5	278.2	208.5	Purpurogenome
241.4	327.3	228.3	
324.2	423.5	329.2	
519.5	581.6	424.9	
	621.9	590.6	
		621.9	

TABLE III

COMPARISON OF UV DATA OF THE PRESENT STUDY WITH THOSE OF THE SAME METABOLITES IN FRISVAD AND THRANE[6] AND COLE AND COX[5]

x = Wavelength taken from the listed maxima and y = wavelength directly from the spectrum provided in Cole and Cox. sh, sm = Shoulder and small peak, respectively, which were determined by visual inspection of spectra and not printed by the spectrophotometer in the present study. B = Assessment of similarity of data determined by only considering genuine maxima in the present study; C = assessment of similarity of data determined by also comparing shoulders and small peaks in the present data to maxima in ref. 6. + = Very similar/identical; − = obvious difference. 1, 2 = Peaks with highest and second highest absorption value. e = End absorption. I = retention index[6].

Metabolite	*I*	*Present*	*Ref. 6*	*Ref. 5*	*B*	*C*
Austdiol	702	201.7[2]	e	204[2]	+	+
		256.4	256[2]	255		
		380.0[1]	378[1]	379[1]		
Brevianamide A	865	200.9[1]			−	+
		232.0[2]	234[1]			
		255sh	254			
		406.2	404[2]			

TABLE III *(continued)*

Metabolite	*I*	*Present*	*Ref. 6*	*Ref. 5*	*B*	*C*
Canescin	907–994	200.1[2]			–	+
		247.3[1]	247[1]			
		280sm	279[2]			
		290sm	289			
		327.4	331			
Carlosic acid	692	199.8			+	+
		230.9[2]	230[2]			
		265.8[1]	263[1]			
Carolic acid	676	196.5			–	–
		205.6				
		230.2[2]	230[2]			
		269.1[1]	263[1]			
Chaetoglobosin C	1174	202.2[1]		212sh	–	–
		221.3[2]	222[1]	223[1]		
			253[2]			
		290sh	280	280		
		295sm	289	292sm		
Citrinin	919	201.3[2]			–	–
		213.7[1]	220[1]	218[1]		
		220sh		220sh		
			235			
		254.6		252[2]		
		320.1	329[2]	319		
Cyclopaldic acid	843	202.3[1]			–	–
		243.9[2]	244[1]			
		285sh	272[2]			
			326			
				x *y*		
Cyclopenin	864	212.0[1]	211[1]	211[1] 215[1]	+	+
		288.8[2]	288[2]	290 290		
				x *y*		
Cyclopenol	779	203.1[1]	211[1]	218[1]	+	+
		281.6	282	285 285		
α-Cyclopiazonic acid	1148	201.3[2]	e[1]	210sh	–	–
		224.5[1]	222[1]	225[1]		
		253sh				
		281.6	279[2]			
		291sh	288	290[2]		
Dehydrocarolic acid	678	199.6			+	+
		244.1[2]	245[1]			
		285.6[1]	294[2]			
Desacetylpebrolide	902	201.5[1]	e[1]		–	+
		229.6[2]	231[2]			
		272sm	273			
3,5-Dimethyl-6-hydroxyphthalide	901	208.7[1]	206[1]		+	+
		242.4[2]	240[2]			
		312.8	298			
Dipicolonic acid	677	202.6[1]			–	+
		220sm	220[2]			
		269.7[2]	271[1]			

(Continued on p. 216)

TABLE III *(continued)*

Metabolite	*I*	*Present*	*Ref. 6*	*Ref. 5*		*B*	*C*
Duclauxin	1142	201.3				–	–
		232.1	224[1]				
			264[2]				
			320				
			342				
Ethisolide	730	201.3	205			+	+
Frequentin	910	198.9[2]	e[1]			+	+
		231.5[1]	230[2]				
		288.8	292				
Fulvic acid	942	200.7[1]	202[1]			–	–
			230				
		330sh	338				
		376[2]	388[2]				
Gentisyl alcohol	662	202.7[1]				–	+
		223sh	220[1]				
		296.8[2]	290[2]				
Gliotoxin	848	202.6[1]				–	–
			216[1]				
		268.3[2]	267				
				x	*y*		
Griseofulvin	990	196.5				+	+
		212.7[2]	210[2]		212[1]		
		233.1	234	236[2]	238sh		
		251.7	250	252			
		287.9[1]	292[2]	291[1]	290[1]		
			328	324	328sh		
Griseophenone C	945	205.3[1]				–	+
		220sh	220[1]				
		296.1[2]	296				
		340sh	334				
Hadacidin	674	204.1	210			+	+
5′-Hydroxyasperentin	828	201.1[2]				+	+
		216.1[1]	215[1]				
		268.8	266[2]				
		301.3	299				
Hydroxyisocanadesic acid	771	200.1[1]	e[2]			+	+
		225.2[2]	225[1]				
Kojic acid	676	200.4[2]				+	+
		217.5[1]	220[1]	220[1]			
		270.4	267[2]	270			
Lapidosin	914	199.8[1]	215			–	–
		229.1[2]	236				
			278[2]				
		320.2	322				
Lichexanthone	1382	201.1[1]	e[1]			–	+
		241.1[2]	240[2]				
		307.2	307				
		335sh	334				

TABLE III *(continued)*

Metabolite	*I*	*Present*	*Ref. 6*	*Ref. 5*	*B*	*C*
6-Methylsalicylic acid	802	209.7[1]	204[1]		–	–
		237.9sm	240[2]			
		311.0[2]	303			
Monorden	928	201.9[1]			–	–
			215[1]			
		266.4[2]	274[2]			
Mycelianamide	1181	201.6[1]			–	–
			210[1]			
		231.2	229			
		324.2[2]	317[2]			
Mycophenolic acid	984	201.9[2]		*x* *y*	+	+
		215.8[1]	217[1]	217 220[1]		
		250.1[2]	249[2]	249 252[2]		
		305.9	302	304 306		
Norlichexanthone	1008	201.0[1]	202[1]		–	–
		241.3[2]	240[2]			
			266			
		311.1	312			
			346			
Orsellenic acid	746	200.6[2]			+	+
		217.5	213[1]			
		259	256[2]			
		300.0	296			
Palitantin	887	198.5			+	+
		232.0[1]	230			
Patulin	684	200.7		*x* *y*	+	+
		275.7[1]	275	269 275		
Paxilline	1287	199.2[2]		*x* *y*	+	+
		230.5[2]	229[1]	230[1] 229[1]		
		281.6	280	281 280		
Penicillic acid	717	200.7		*x* *y*	+	+
		224.8[1]	226	221 225		
Penitrem A	1331	200.9[1]		*x* *y*	+	+
			210[1]	212		
		234.9[2]	234[1]	233[1] 235sh		
		301.9	290[2]	295 292		
Phoenicin	705	200.7[1]	e[2]		–	–
		217.6[2]				
		261.6	267[1]			
		492.8	435			
PR-Toxin	820	201.5[1]			+	+
		247.5	248	249(ethanol)		

(Continued on p. 218)

TABLE III *(continued)*

Metabolite	*I*	*Present*	*Ref. 6*	*Ref. 5*	*B*	*C*
Purpurogenone	1191	201.9[2]	201[1]		–	–
		219.5[2]				
		241.4	250[2]			
		265sm	265			
		310sm	308			
			389			
		485sm	499			
		519.5				
			530			
		560sm				
2-Pyruvoylaminobenzamide	767	211.2[1]	210[1]		–	–
		248.0[2]	236			
		301.3	306[2]			
Roquefortine B	674, 694	202.2[2]		212sh	–	+
		223.7[1]	223[1]	220[1]		
		275sh	275	275sh		
		281.9	280[2]	280		
		290sm	289	290sm		
Roquefortine C	928	203.1[1]	206[2]	209[1]	–	–
		238.6	232	238		
		324[2]	304[1]	328[2]		
Roseopurpurin	869	201.0[2]			–	–
		218.5[1]	210[1]			
		248.3	248			
			267			
			278			
		285.7	284[2]			
		429.6	434			
Rugulovasine A	724	203.6[2]			–	+
		221.2[1]	220[1]	224[1]		
		287.2	284[2]	284sm		
		290sh	290	290		
Scytalone	723	200.7[2]	e[1]		–	+
		221.3[1]	230			
		282.1	282[2]			
		320sh	315			
Secalonic acid D	1176	201.1[1]			–	–
			220[1]	220sh		
		240sh	248	242		
		340.8	334[2]	346		
Spinulosin	707	210.4[1]	e	210[1]	+	+
		301.1	294[1]	294		
Sterigmatocystin	1100	201.9[2]	208[1]	210[2]	–	+
		230sh	232	232sh		
		246.4[1]	246[2]	246[1]		
		326.5	324	326		
Stipatic acid	693	200.1[2]			–	+
		263.0[1]	258[1]			
		324.1	334			
		369.1	358[2]			

TABLE III *(continued)*

Metabolite	*I*	*Present*	*Ref. 6*	*Ref. 5*	*B*	*C*
Terrestric acid	738	201.3[2]	e		+	+
		228.5[1]	230[2]			
		272.9	272[1]			
Viomellein	1234,1246	200.7[1]		*x*	−	−
		222.4	222[2]	225[2]		
		264.1[2]	263[1]	264[1]		
			298			
		380.3	374	395		
Viridicatum toxin	1200	201.2[1]	e[1]		−	−
		235.2	241[2]	238[2]		
		283.2[2]	283	280		
			325	325sh		
			330			
			349	345sh		
		449.6	431	430		
Xanthocillin	201.1[1]				Not compared	
(four peaks by HPLC)	225sm		218[1]			
			235			
	240sh		240			
			252			
	295sh		295[1]			
			327[2]			
	346		348[2]			
			370[2]			
			371			

study but not in ref. 6, and a maximum was recorded in ref. 6 but only a shoulder was obtained in present study; duclauxin peaks at 224, 320 and 342 nm in ref. 6 are missing in the present study and the mycelianamide peak at 210 nm in ref. 6 is missing in the present study; norlichexanthone peaks at 266 and 346 nm in ref. 6 are missing in the present data; etc. (Table III).

TABLE IV

PERCENTAGE OF METABOLITES WHICH HAVE VIRTUALLY IDENTICAL/IDENTICAL UV SPECTRAL DATA IN THE PRESENT STUDY AND IN FRISVAD AND THRANE

B, C as for Table III; n = number of metabolites; I = retention index.

I	n	*B (%)*	*C (%)*
600–699	11	54	91
700–799	12	67	83
800–899	9	44	56
900–999	11	27	54
1000–1099	1	0	0
1100–1199	7	0	14
1200–1299	3	33	33
1300–1399	2	50	100

The variation in the data at both the lower and higher *I* values, where the solvent composition was the same or most similar, can be explained by the different solvent systems used in ref. 6 and the methanol used here. However, the increase in variation observed at the intermediary *I* values is probably due to the changes in solvent composition and especially the large pH decreases which would result from the increasing concentrations of trifluoroacetic acid used in the gradient[6].

The use of UV spectra obtained by gradient HPLC–DAD to indicate biosynthetic relatedness of metabolites from similarity of chromophores, and hence their taxonomic value in fungi, has been recommended[6,7]. However, it has been indicated here that misidentification of metabolites and chemosyndromes and consequently erroneous use of the data in systematics on the basis of similar UV maxima as determined by gradient HPLC–DAD could occur; spectra with similar UV maxima need not have similar chromophores, and dissimilar UV maxima could be from similar chromophores, especially if the metabolites exhibit large differences in *I* by gradient HPLC–DAD as in ref. 6. The effect of solvent compositions on UV spectra used in HPLC–DAD merits further investigation in view of these findings. The solvent system developed by Paterson and Kemmelmeier[2] might be more suitable for the purpose of chromophore comparison and fungal chemotaxonomy by HPLC–DAD than those used in refs. 6 and 7, as the system does not contain an acidic component and large pH changes would not occur.

UV spectra are useful for the identification of metabolites, and additional characteristic information is provided by the difference spectra. These data can be of value, for example, in the further characterization of spots removed from TLC or PLC plates[1]. If gradient solvent DAD systems are used in HPLC, the solvent is not uniform throughout and conditions for taking the spectra could be different at different stages of the gradient depending on the construction of the gradient. As indicated here, this might cause difficulties in the identification of compounds by UV absorption, particularly where large pH changes occur in the solvent because of the chosen gradient.

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