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The Xylariaceae: A Case Study in Biological and Chemical Diversity

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Abstract: filamentous fungi have an excellent track record regarding their ability to synthesize a diverse range of metabolites which often possess potent biological activity. However with the exception of a few genera they have been much neglected and with a world estimate of 1.5 million species there is little chance of short supply for future investigations. The ascomycete family, the Xylariaceae, is well known for the wide biological diversity of many of its species and genera. It has also been found to be the source of an impressive array of metabolites of which many have proved to be novel structures. The presence or absence of specific chemicals can in many cases be linked with taxonomic position and provides a powerful tool for systematic studies and for the identification of problem taxa. The variety of novel chemicals recognized to date is impressive and with many new taxa awaiting investigation the future potential of the family is considerable.

INTRODUCTION

The fungi as producers of chemicals

"The terrestrial mycelial fungi amongst the eukaryotes, and the actinomycetes amongst the prokaryotes, share a number of physiological and ecological features. The filamentous morphology of both groups is associated with the efficient utilization of exogenous, macromolecular, and often insoluble substrates, as well as the ability to produce a remarkably diverse range of secondary metabolites" (1). Furthermore fungal secondary metabolites have a great diversity of molecular structure and frequently show taxonomic specificity in their production which usually occurs during the stationary phase of growth or the idiophase (2). "The best known secondary metabolites have medicinal, industrial, or agricultural impact as antibiotics, toxins, anticancer drugs, dyes, growth promoters, hallucinogens,

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immunosuppressants and so on" (3). Without doubt the fungi are prolific producers of secondary metabolites. Turner (4) recorded almost 1000 fungal products in 1971 but by 1983 this had more than doubled (5) and today it is realistic to anticipate in excess of 6000 different metabolites produced by fungi. Not only do fungi exhibit an impressive output but amongst their products are some of the most important commercial compounds. The antibiotic penicillins and cephalosporins, and the immunosuppressant cyclosporin B are widely perscribed drugs and rank in the top 5% selling pharmaceutical products (6, 7)

The untapped resources of the fungi

On the basis of an estimated 1.5 million species, the number of fungi in the world represents, after the insects and probably the bacteria, one of our greatest but under utilized living resources (8). At present around 72,000 species of fungi have been described which indicates that only about 5% of the world's fungi have as yet been recognized. There have, however, been suggestions that this prediction of 1.5 million species is too high and a working figure of 1 to 1.5 million species is widely accepted (9, 10, 11). In contrast it can be argued that the figure of 1.5 million is conservative. Smith and Waller indicated that there could be 1 million undescribed fungi on tropical plants (12) and Cannon extrapolating from his knowledge of the Phyllachoraceae strongly supported a much higher figure (13). Regardless of the true number of fungi they must certainly be seen as an enormous untapped resource available to the pharmaceutical industry.

THE XYLARIACEAE: BIOLOGICAL DIVERSITY

The Xylariaceae (Xylariales, Ascomycotina) is a large family comprising of around 40 genera (14, 15, 16) and although it has representatives in most countries of the world the Xylariaceae exhibits its greatest diversity in the tropics (16). As a result of comparatively recent in depth taxonomic studies, including biochemical, cultural and chemical approaches, there is now a reasonable understanding of species boundaries and intergeneric relationships within the family (15, 16, 17). In early taxonomic studies in the Xylariaceae investigators were often confused by the wide variation in morphological form exhibited by many of the taxa. An extreme case being that of the dimorphic species *Camillea leprierii* Mont. In its upright or 'camilleoid' form it was placed in *Camillea* Fr. Whilst in its expanded or 'hypoxyloid' form it was referred to *Hypoxylon* Bull. as *H. melanspis* Mont. (18). The considerable variation in gross stromal morphology in certain species of *Xylaria* has in the past resulted in the same species being described as separate taxa (19, 20). Furthermore, a lack of modern collections of tropical species has been an additional problem when attempting to understand diversity in certain genera. In developing a modern systematic arrangement within the family teleomorphic features such as stromal form and colour, type of ostiole, structure of the ascus apical apparatus, shape and dimensions of ascospores, presence or absence and position of the germ slit, presence or absence of spore wall ornamentation have all proved to be useful taxonomic characters (15, 16, 21). Examination of the anamorphs, when these are produced, has also provided a valuable source of taxonomic characters which in the past were badly neglected (21, 22, 23). In the absence of a realistic inventory of the Xylariaceous genera it is still not possible to accurately assess the number of existing species or to predict with confidence how many might be expected. However on the basis of the recent revisions of *Camillea* (18) *Daldinia* (24) and *Hypoxylon* (15) and a series of accounts of *Xylaria* from different regions (25, 26, 27) it is possible to provide some indication of likely numbers for these Xylariaceaous genera.The figures for the number of known species are based on those provided in the current Dictionary of the Fungi (28) subject to modifications for recently revised genera. The predicted number of species takes into account recent descriptions of new species and expectations for further new species predicted on the basis of percentage new species described following recent explorations in the tropics (29, 30).

Table 1. Major genera of the Xylariaceae

Genus	Number of known species	Predicted Number of species
Anthostomella Sacc.	50	?100
Biscogniauxia Kuntze	25	40+
Camillea Fr.	30	40+
Daldinia Ces. & De Not.	17	20+
Hypoxylon Bull.	126	200+
Rosellinia De Not.	100	?
Xylaria Hill ex Schrank	100	?500+

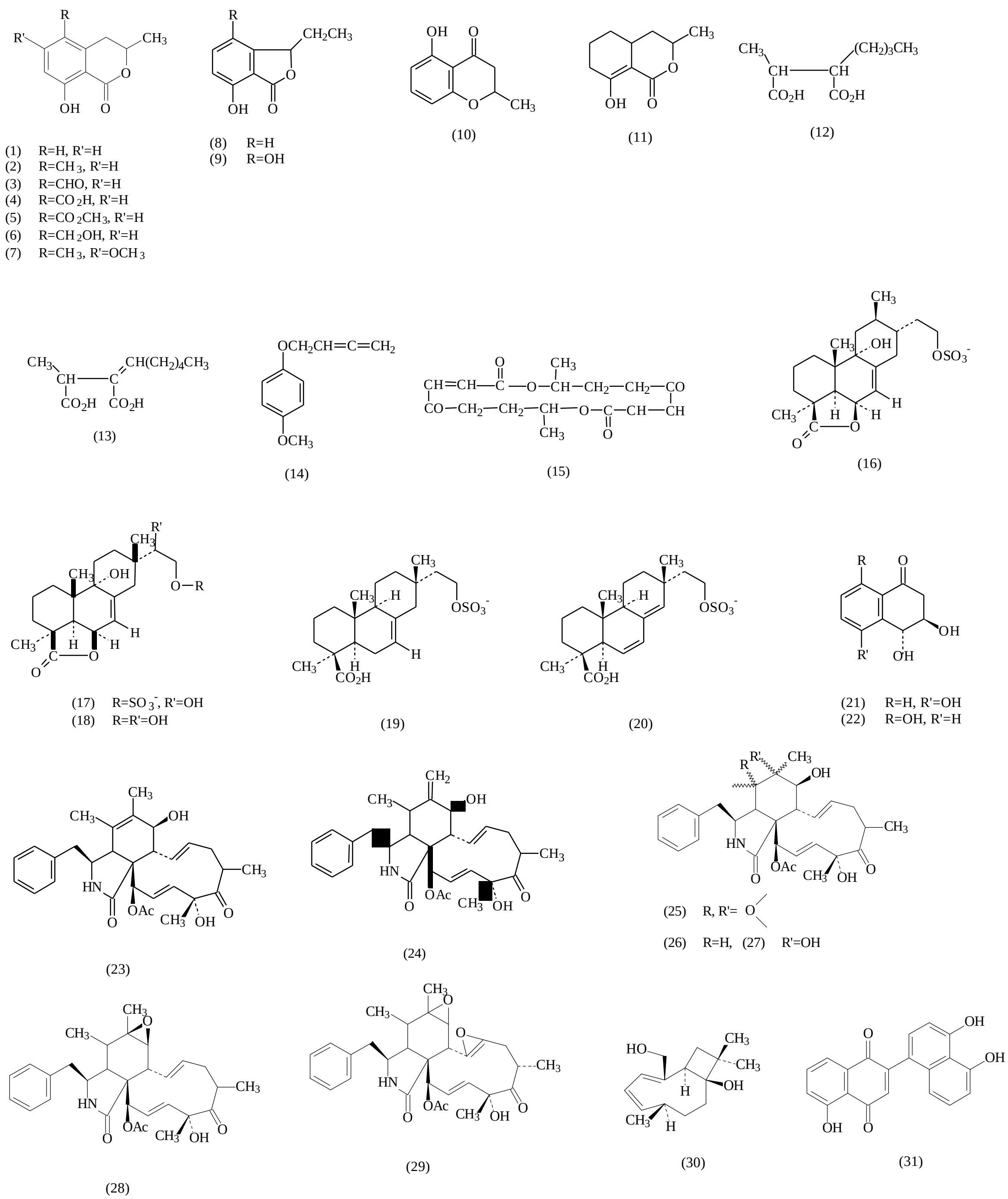
These figures will undoubtedly change as further surveys are carried out in previously under recorded regions, especially in the tropics. Following a three year survey of the Xylariaceae in Thailand Thienhirun (31) found that over 50% of *Hypoxylon* species which she recorded were new and a similar figure for *Xylaria* appears probable. The recent discovery of *Camillea* selangorensis M.A. Whalley, Whalley & E.B.G. Jones in Malaysia (32) together with the more recent finding of 2 further new species was totally unexpected. The genus *Camillea* has always been considered to be mainly restricted to the forests of South and Central America with C. tinctor (Berk.) Laessoe, J.D. Rogers and Whalley as the only previously recorded species from Asia (18, 33). There would appear, therefore, to be a considerable number of unknown species of Xylariaceae in tropical South East Asia!

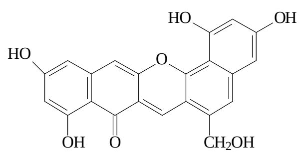
THE XYLARIACEAE: CHEMICAL DIVERSITY

Unlike most ascomycete genera the Xylariaceae has received considerable attention over the past 25 years regarding production of secondary metabolites (17}. In the earliest investigations of the family *Daldinia concentrica* (Bolt.:Fr.) Ces. & De Not. was found to contain 4,9-dihydroxyperylene quinone in the ascocarps (34) whilst 1,8-dimethyoxynaphthalene and its corresponding ether were produced in culture (35). During the same period Chen (36, 37) isolated rosellinic acid and diketopiperazine from cultures of *Rosellinia necatrix* Prill. and subsequently it was shown to produce cytochalasin E (38). Engleromycin, an epoxide of cytochalasin D was later isolated from the xylariaceous taxon, *Engleromyces goetzii* P. Henn. (39). More recently *Hypoxylon fragiforme* (Pers.:Fr.) Kickx was found to owe its orange to brick red stromal colour to mitorubrin and its derivatives (40) whilst *Xylaria polymorpha* Pers. produces a hydroxyphthalide derivative, xylaral, which develops a violet purple colour with aqueous ammonia (41). Extensive studies in our laboratories have resulted in the characterization of many secondary metabolites from a range of representatives of the family and have demonstrated a remarkable diversity of chemical compound produced. A considerable number of these metabolites have proved to be new (17).

The major metabolites produced by the representatives investigated can be grouped as dihydroisocoumarins and derivatives (42), succinic acid and derivatives (43, 44), butyrolactones (45, 46), cytochalasins (47), sesquiterpene alcohols (punctaporonins) (48, 49), griseofulvin and griseofulvin derivatives (17), naphthalene derivatives (17), and long chain fatty acids (50, 51) . Generally the presence of these compounds (Fig. 1) can be seen to be closely related to systematic position (Table 2) and chemical data has proved invaluable in recognizing associations between species, species groups, and genera (16, 17).

Figure 1. Selected secondary metabolites from the Xylariaceae





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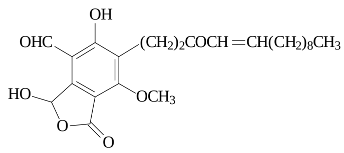

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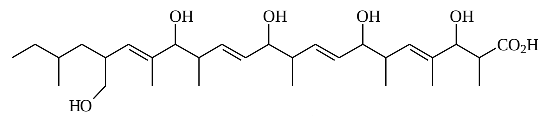

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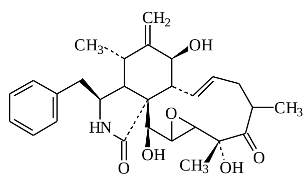

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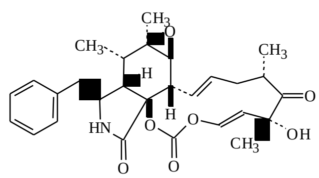

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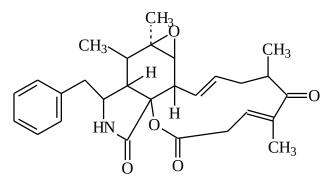

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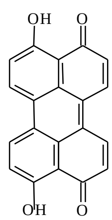

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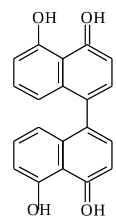

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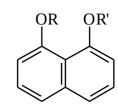

(40) R=H, R'=CH_3
(41) R=R'=CH3

(1) Mellein. (2) 5-methyl-mellein. (3) 5-formyl-mellein. (4) 5-carboxy-mellein. (5) 5-methoxycarbonyl-mellein. (6) 5-hydroxymethyl-mellein. (7) 6-methoxy-5-methyl-mellein. (8) Iso-ochracein. (9) 4-hydroxyiso-ochracein. (10) Chromanone. (11) Ramulosin. (12) 2-butyl-3-methylsuccinic acid. (13) 2-hexylidene-3-methylsuccinic acid. (14) 4-(4`-methoxyphenoxy)-buta-1,2-diene. (15) Pyrenophorin. (16) Hymatoxin A. and its derivatives (17) B, (18) C, (19) D, (20) E. (21) 3,4,5,-trihydroxynaphthalenone. (22) 3,4,8,-trihydroxynaphthalenone. (23) Cytochalasin C. (24) Cytochalasin D. (25) Cytochalasin N. (26) Cytochalasin O. (27) Cytochalasin P. (28) Cytochalasin Q. (29) Cytochalasin R. (30) Punctaporonin B. (31) Hypoxylone. (32) Hypoxyxylerone. (33) Xylaral. (34) Cubensic acid. (35) Engleromycin. (36) Cytochalasin E. (37) Rosellichalasin. (38) 4,9-dihydroxyperylene quinone. (39) binaphthyl (40 & 41) Naphthalene derivatives.

Table 2. Generic distribution of major metabolites

	1	2	3	4	5	6	7	8	9	10	11
Butyrolactones							+				
Cytochalasins						+				+	+
Dihydroisocoumarins	+	+			+						0
Succinic acid and derivatives											+
Naphthalenes			+	+							
Sesquiterpene alcohols								+			
Griseofulvin and derivatives									+		

1. *Biscogniauxia*. 2. *Camillea*. 3. *Daldinia*. 4. *Entonaema*. 5. *Hypoxylon*. 6. *Kretzschmaria*. 7. *Nemania* 8. *Poronia*. 9. *Thamnomyces* 10. *Rosellinia*. 11. *Xylaria*.

+ = consistently present*; 0* = occasionally present

The dihydroisocoumarins are widely distributed throughout the family but are probably more representative of *Hypoxylon*, *Biscogniauxia* and *Camillea* (17). Butyrolactones so far appear to be restricted to *Nemania serpens* (Pers.:Fr.) Pouzar whilst cytochalasins are more frequently encountered in species of *Xylaria*, *Rosellinia* and members of the now defunct section Primo-cinerea of *Hypoxylon* (16).

FUTURE POTENTIAL

New isolates

Although it is difficult to estimate the percentage of taxa of Xylariaceae which have been grown in artificial culture it is likely to be considerably higher than the figure for fungi in general. This is mainly a result of the work of Martin in the 1960's (21), Rogers and his students over the past 25 years (e.g. 15, 22, 24, 25, 26, 27), Van der Gucht and Van der Veken (52), and our own studies on metabolites (16, 17). In the recent revision of *Daldinia* 17 species were recognised of which 7 (41%) were cultured (24) and for *Hypoxylon* 67 or 53% of the 126 species accepted have been cultured (15). These are the 2 genera which have received most attention but in most genera a much smaller percentage of the known species has been cultured. We suggest that probably 2/3 or more of all known Xylariaceae are still to be cultured.

New species

Recent investigations of the family in different tropical regions all indicate a large number of undescribed species. In an account of *Xylaria* from the Cerro de la Neblinia in Venezuela about 37% of the taxa examined were unknown (25) and roughly half of the taxa described had not been included by Dennis in his account of the family in Venezuela and surrounding countries (53). Likewise in a study of *Xylaria* of Mexico 28% of the taxa found proved to be unknown

(27). Similar indications of considerable numbers of undescribed species are known for *Hypoxylon* in Thailand with approximately 50% believed to be new (31) and investigations of the Xylariaceae from Sulawesi and Papua New Guinea confirm that there are a significant number of new taxa in those regions (54).

Endophytes

The Xylariaceae are almost ubiquitous, and sometimes dominant, endophytic inhabitants of living plants (55) and occur as major representatives in most tropical plants which have been investigated (56). *Xylaria*, in particular, appears to be commonly represented (55, 56). A problem occurs concerning the identification of many of the xylariaceous endophytes since the presence of an anamorph does not always provide sufficient information to allow confident identification. Furthermore in many isolates anamorph forms are not produced in culture (55). Current studies on endophytes of teak leaves in northern Thailand reveal an exceptional high occurrence of the Xylariaceae with *Xylaria* providing in some samples over 60% of the total. It has recently become possible to identify some of these isolates by comparing metabolite profiles of endophytic isolates with those obtained from free living teleomorphic, and thus identified, forms. Other endophytes have been identified by inducing teleomorph formation through inoculation onto presterilized branches followed by incubation under simulated field conditions (57). Preliminary examination of endophytic *Xylaria* isolates from teak indicate the presence of at least 10 different species including several which produce novel metabolites.To what extent these are the products of unknown, or not yet investigated species, or are products only produced by endophytic forms of a known free living species is unclear. Regardless of their true taxonomic status many xylariaceous endophytes produce an interesting array of metabolites including those with novel structures.

CONCLUSIONS

The Xylariaceae with their worldwide distribution, variation in form, and remarkable ability to produce a wide range of diverse chemicals offer an interesting challenge for both the mycologist and the chemist. Certainly they are a group of fungi where collaboration with natural products chemists has proved invaluable in confirming systematic associations based on traditional mycological approaches and on the basis of chemical profiles provides a means to identifying certain taxa in the absence of suitable morphological characters. Their ability to produce novel compounds continues to provide the chemist with interesting, and frequently unusual, structures. The genus *Xylaria* has proved to be one of the most rewarding with 30 new compounds isolated from 30 species. In common with views on most secondary metabolites their raison d’etre remains unknown although cytochalasin E might be implicated in the ability of *R. necatrix* to be phytopathogenic (16).

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