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ANTIMICROBIAL PROPERTIES OF UMBELLIFERONE DERIVATIVES*

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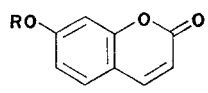
Abstract—The inhibitory effects of umbelliferone and a number of its alkyl and acyl derivatives on the growth of a variety of bacteria, yeasts and molds have been examined. Herniarin and similar alkyl ethers are more effective antimicrobial agents than umbelliferone.

INTRODUCTION

THE RESISTANCE of plants to disease may be due to the presence of preformed natural fungicides, or to the synthesis of these compounds (phytoalexins) in the plant tissue in response to fungal or viral infection.[1] Wain[2] recently suggested that further identification of the structures and properties of natural protective agents should lead to the recognition of new, effective, and perhaps, safer agricultural fungicides. With this objective we have now investigated the inhibitory effects of some classes of natural fungicides and their derivatives on the growth *in vitro* of a representative variety of microorganisms, particularly species involved in food spoilage.

Although it has been suggested that some coumarins, including umbelliferone, scopoletin, and furanocoumarins, play important fungicidal roles in plants,[2,3] the activities of these substances against a wide variety of bacteria, yeasts and molds have not been extensively studied, and little is know of the effects of structural variations within this class on their microbial inhibition. In the case of fungi it has been shown that coumarin inhibits growth of the yeast *Saccharomyces cerevisiae*,[4] and at higher concentrations (100–1000 ppm) inhibits or retards germination of the spores of *Aspergillus niger* and *Penicillium glaucum*.[5]



(I)

Ia, R = H; b, R = Me; c, R = Et;
d, R = Pr; e, R = allyl; f, R = 3-methylbutyl;
g, R = 3-methylbutenyl; h, R = geranyl;
i, R = benzyl; j, R = cinnamyl; k, R = acetyl;
l, R = propionyl; m, R = butyryl,
n, R = myristyl; o, R = benzoyl

* Part III in the series "Antimicrobial Properties of Natural Phenols".

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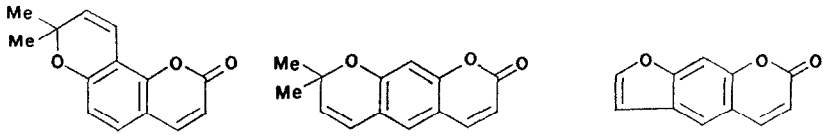
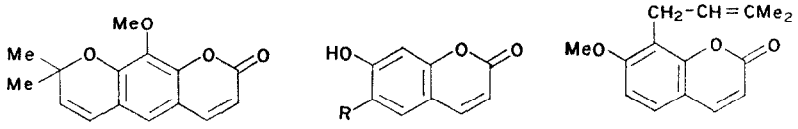


(II) (III) (IV)

Chakraborty *et al.*[6,7] studied the activity of umbelliferone (7-hydroxycoumarin Ia) and of sixteen other natural coumarins at concentrations of 2×10^{-2} M to 2×10^{-5} M against *Aspergillus niger* and *Curvularia lunata* and found that umbelliferone was virtually inactive against these fungi at lower concentrations. Seselin (II), xanthyletin (III), psoralen (IV) and luvangetin (V), however, markedly inhibited growth of *C. lunata*, and to a lesser extent, of *A. niger* at concentrations between 30 and 300 ppm.

Umbelliferone has been reported to be active against *Brucella* species.[8–10] However, from a recent examination of the effects of umbelliferone and other natural coumarins on the growth of Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus lysodeicticus*, and *Bacillus megatherium*) and Gram-negative bacteria (*Escherichia coli*, *Aerobacter aerogenes* and *Serratia marcescens*), Dadák and Hodák[11] reported that umbelliferone (5×10^{-3} M) is completely inactive against all of these microorganisms. Gram-negative bacteria were not affected by any of the coumarins they examined. However, ostruthin



(V) (VI), R=CH_2-CH=C(Me)-$(CH_2)_2$-CH=CMe_2 (VII)

(VI) strongly inhibited growth of the Gram-positive bacteria at concentrations of 10^{-4} M to $2{\cdot}5 \times 10^{-4}$ M, and it was also[12] effective against the yeast *Saccharomyces cerevisiae*. These authors suggested that the antibacterial activity of ostruthin requires both the free 7-hydroxyl group and the long geranyl side-chain, since methylation of the hydroxyl resulted in considerable loss of activity. In contrast to this observation, however, it has been reported[13] that the 7-methoxycoumarin, osthol (VII), is very active against some species of Gram-positive bacteria.

RESULTS AND DISCUSSION

With the exception of ostruthin, umbelliferone derivatives which have been reported to show activity possess a protected 7-hydroxyl group. In this communication additional studies on the effects of *O*-alkylation and of *O*-acylation on the inhibitory activity of umbelliferone are reported. To obtain comparative structure–activity relationships, growth inhibi-

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TABLE 1. EFFECT OF 500 ppm OF UMBELLIFERONE DERIVATIVES ON GROWTH OF FUNGI

Coumarin	*Zygosaccharomyces japonicus* C124	*Zygosaccharomyces barkeri* C293	*Candida tropicalis* C147	*Candida chalmersi* C127	*Pichia chodati* var. *fermentans* C238	*Hansenula anomala*	*Saccharomyces cerevisiae* var. *ellipsoideus* Y44	*Saccharomyces rosei* 59–4	*Saccharomyces mellis*	*Torula utilis* NRRL Y660	*Hanseniaspora melligeri* C23	*Aspergillus flavus* NRRL 3145	*Aspergillus flavus* NRRL 2999	*Aspergillus niger* A-7705	*Aspergillus oryzae*	*Aspergillus glaucus*	*Byssochlamys fulva* NRRL 3493	*Penicillium chrysogenum* 52	*Rhizopus senti NRRL* 2868	*Botrytis cinerea* NRRL 3492	*Alternaria* spp.
Ia	−	−	−	−	−	−		−	−		−	−	−	−	−	−	+	−			
Ib	+	+	+	+	+	−		+	+		+	+	+	+	+	+	+	+			
Ic	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+			
Id	+		+		+	−	−			−		−		−			±	+	−	+	−
Ie	−		−		−	−						−		−			+	−			
If	−		−		−	−	−			−		−		−			−	−	−		
Ig	−		−		−	−	−			−		−		−			−	−	−		
Ih	−	−	−	−	−	−		−			−	−	−	−	−	−	−	−			
Ii	−	−	−	+	−	−		−	−		−	−	−	−	−	−	+	−			
Ij	−		−		−	−	−		+	−		−		−				−	−	−	−
Ik	+		+		+	+	+			+		+		+			+	+	−		
Il	+		+		+	+	+			+		+		+			+	+		+	+
Im	+		+		−	−	−			−		−		−			−	−	−	+	+
In	−		−		−	−	−			−		−		−			−	−	−		
Io	−		−		−	−				−				−			+	−			
VIII	−		−		−	−	−			−		−		−			±			±	±
IXa	−		−		−	−	−			−		−		−			−	−	−	−	−
IXb	−		−		−	−	−			−		−		−			−	−	−	−	−

The results are expressed on the basis: + = complete growth inhibition. Measured after 4–5 days. ± = not completely effective; faint growth occurs. − = ineffective; growth occurs.

tion was measured by Lederberg's[14] replica plating technique with a coumarin concentration of 500 ppm.

The effects of umbelliferone derivatives on the growth of fungi are summarized in Table 1. With the exception of *Byssochlamys fulva*, umbelliferone (Ia) was ineffective against the wide variety of yeasts and molds shown in the table. In contrast to the inactivity of umbelliferone, its methyl ether (Ib), herniarin, completely inhibited the growth of five *Aspergillus* species, of *Byssochlamys fulva* and *Penicillium chrysogenum*, and of eight of the nine species of yeasts examined. Herniarin is a constituent of some food plants and was recently found[15] in the leaves of celery which had been attacked by *Septoria apii*. The broad-spectrum,

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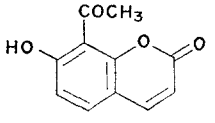
[15] G. CAVALIE, *Compt. Rend.* **258** (2), 689 (1964); *Chem. Abs.* **60**, 12384 (1964).

antifungal properties of herniarin provide additional evidence that this substance may be an important, natural protective agent for celery and other plant species.

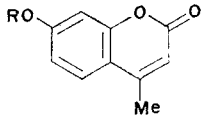
The inhibitory effect of herniarin demonstrates that the antifungal activity of umbelliferone may be increased by simple alkylation of the 7-hydroxyl group. The extent to which increased antifungal activity is dependent on the length of the alkyl side-chain and on its degree of saturation is indicated by comparison of the inhibitory effects of similar alkylated derivatives. Thus, 7-ethoxycoumarin (Ic) is similar to herniarin, and it completely inhibited growth of all fungi examined, including the yeast *Hansenula anomala* which was not affected by herniarin. 7-Propoxycoumarin (Id) is less broadly effective than the ethyl and methyl derivatives, and it inhibited the growth of only six of the thirteen species of fungi used. 7-Allyloxycoumarin (Ie) was active only against *Byssochlamys fulva*, and is much less effective, therefore, than the saturated analogue (Id). 7-(3-methylbutyl)oxy-,7-(3-methylbutenyl)oxy-, and 7-geranyloxycoumarin were inactive against all species of fungi. On the basis of these results and the earlier observations of Chakraborty *et al.*[6,7] it is clear that the antifungal activity of umbelliferone may be increased by *O*-alkylation with saturated, lower alkyl groups (ethyl > methyl > propyl > isopentyl), and by the formation of cyclic ethers of types (II)–(V). On the other hand, alkenyloxy derivatives such as 7-(3-methylbutenyl)oxy-, 7-geranyloxy-, 7-farnesyloxy-, and 7-allyloxycoumarin, some of which occur naturally, are as inactive (at 500 ppm) as umbelliferone itself.

It is of interest that the effects of *O*-acylation on the antifungal activity of umbelliferone are similar to those observed upon *O*-alkylation. Thus, 7-acetoxycoumarin (Ik) and 7-propionyloxycoumarin (Il) inhibited the growth of nine and eleven species of fungi, respectively (Table 1). Activity drops with increasing length of the acyl group. 7-Butyryloxycoumarin (Im) was active against only four species, while the myristoate (In) and benzoate (Io) were generally inactive.

Alkylation and acylation of umbelliferone with lower alkyl and acyl groups increase its inhibitory effect on the growth of a number of species of bacteria. Thus, umbelliferone (Ia) and its allyl, 3-methylbutyl, 3-methylbutenyl and geranyl ethers were completely ineffective against the Gram-positive and Gram-negative bacteria shown in Table 2. Herniarin (Ib), however, inhibited the growth of *Bacillus cereus*, *Sarcina lutea*, and *Alcaligenes faecalis*, while 7-ethoxycoumarin inhibited growth of five species, including the Gram-negative organisms, *Escherichia coli* and *Salmonella typhimurium*. 7-Acetoxycoumarin (Ik) exhibited



(VIII)



(IXa), R = H
(IXb), R = Me

a bacteriostatic effect on the growth of *Bacillus cereus* and of the five Gram-negative microorganisms for a period of 48 hr, after which period normal growth of the organisms occurred. A similar bacteriostatic effect was observed with the propionate (Il). The nuclear-substituted umbelliferone derivatives (VIII), (IXa) and (IXb) were generally inactive against both bacteria and fungi.

Measurements of the approximate minimal inhibitory concentrations of herniarin and the other active, umbelliferone derivatives described above indicate that, with a few excep-

TABLE 2. EFFECT OF 500 ppm OF UMBELLIFERONE DERIVATIVES ON GROWTH OF BACTERIA

Coumarin	*Bacillus cereus* 2006	*Sarcina lutea*	*Staphylococcus aureus* SG8A	*Streptococcus lactis*	*Alcaligenes faecalis* B170	*Escherichia coli* ML30	*Pseudomonas aeruginosa* 111	*Salmonella typhimurium* Tml	*Serratia marcescens*
Ia	−	−	−	−	−	−	−	−	−
Ib	+	+	−	−	+	−	−	−	−
Ic	+	−	−	+	+	+	−	±	−
Id	−	±	±	−	−	−	−	−	−
Ie	−	−	−	−	−	−	−	−	−
If	−	−	−	−	−	−	−	−	−
Ig	−	−	−	−	−	−	−	−	−
Ih	−	−	−	−	−	−	−	−	−
Ii	−	−	−	−	−	−	−	−	−
Ij	−	−	−	−	−	−	−	−	−
Ik*	±	−	−	−	±	+	±	±	±
Il*	±	−	−	±	−	±	−	±	±
Im	−	−	−	−	−	−	−	−	−
In	−	−	−	−	−	−	−	−	−
Io	−	−	−	−	−	−	−	−	−
VIII	+	−	−	−	−	−	−	−	−
IXa	+	−	−	−	−	−	−	−	−
IXb	−	−	−	−	−	−	−	−	−

* Measured after 48 hr. Growth occurs thereafter. + = complete growth inhibition. ± = not completely effective; faint growth occurs. − = ineffective; growth occurs.

tions, these compounds are not effective microbial inhibitors at concentrations substantially less than 500 ppm. Exceptions noted include 7-propoxycoumarin which is active against *Byssochlamys fulva* and *Botrytis cinerea* at 125 ppm. 7-Acetoxy-, 7-propionyloxy-, and 7-butyryloxycoumarin were active against *Botrytis* cinerea at 125 ppm, and against the yeasts *Zygosaccharomyces japonicus* and *Candida tropicalis* at 250 ppm.

On the basis of the above results it is apparent that umbelliferone and its derivatives are not sufficiently active at low concentrations to be considered as potentially useful preservatives. Herniarin and similar alkylated derivatives, however, may be effective protective fungicides in plants because of their localized formation in high concentrations at the point of infection.

EXPERIMENTAL

The coumarins used in this investigation are mostly known compounds. They were synthesized from umbelliferone by standard alkylation and acylation procedures. Purity and identity of each compound was confirmed by elemental analyses and by means of its NMR spectrum.

All compounds were initially tested at a concentration of 500 ppm (w/v). Plates were prepared by adding a measured amount of the coumarin in acetone solution to 10 ml of sterilized medium, mixing thoroughly, pouring into 60 × 15 mm plastic Petri dishes, and allowing the gel to set. The plates were then inoculated with the test organisms. In the case of bacteria and yeasts the inoculation was done by Lederberg's replica plating technique,[14] applying nine bacteria or seven yeasts on each plate. In the case of molds, drops of homogenized culture were placed on the surface of the plates, applying three of four molds per plate. The media used were: plate count agar (Difco) (pH 7·0) for bacteria; potato dextrose agar (Difco) (pH 5·6) for yeasts and molds. Control plates were also prepared containing the media plus acetone and inoculated with the same organisms. The plates were incubated at 28° for 1–5 days and evaluated by comparison with the controls.

Sources of the cultures used in screening are identified in Tables 1 and 2.