Phytochemistry ,1971, Vol. 10, pp. 2971 to 2974. Pergamon Press. Printed in England.

SHORT COMMUNICATION

ANTIMICROBIAL PROPERTIES OF 6,7-DIHYDROXY-, 7,8-DIHYDROXY-, 6-HYDROXY- AND 8-HYDROXYCOUMARINS*

L. JURD, J. CORSE, A. D. KING, JR., H. BAYNE and K. MIHARA

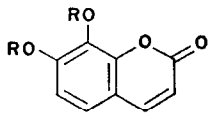
Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710, U.S.A.

(*Received* 16 *March* 1971)

Abstract—The effects of daphnetin, aesculetin, scopoletin, 6-hydroxycoumarin and 8-hydroxycoumarin and their alkyl and acyl derivatives on the growth of bacteria and fungi are reported.

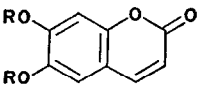
INTRODUCTION

DAPHNETIN, 7,8-dihydroxycoumarin (Ia), and its isomer, aesculetin, 6,7-dihydroxycoumarin (IIa), occur free in *Euphorbia, Daphne* and *Fraxinus* species, and as glycosides or partially methylated derivatives in other plant species.[1] Scopoletin (IIIa) normally occurs only in trace amounts in the tissues of healthy plants (tobacco, potato) of the Solanaceae. However, its concentration increases significantly around the lesions resulting from virus infection[2,3] and it is considered to be a natural plant fungicide.[4] Both scopoletin and aesculetin have been detected in celery leaves attacked by *Septoria apii.*[5]



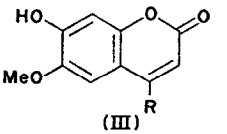
(I)

Ia, R = H; b, R = Me; c, R = allyl; d, R = acetyl.



(II)

IIa, R = H; b, R = Me; c, R = acetyl.



(III)

IIIa, R = H; b, R = COOH; c, R = COOEt.

Studies on the broad, antimicrobial effects of daphnetin, aesculetin and their derivatives are limited. In 1962, Grebus *et al.*[6] investigated the effects of daphnetin, daphnetin 7-glucoside, aesculetin and aesculetin 6-glucoside on the growth of the bacteria *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.* Aesculetin and the two glucosides were completely inactive. Daphnetin (Ia), however, was effective against *S. aureus* and *E. coli* at a concentration of 200 ppm and against *P. aeruginosa*

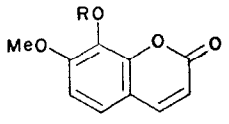
* Part IV in the series "Antimicrobial Properties of Natural Phenols".

[1] F. M. DEAN, *Naturally Occurring Oxygen Ring Compounds*, pp. 187–89, Butterworths, London (1963).
[2] R. J. BEST, *Austral. J. Exptl Biol. Med. Sci.* **22**, 251 (1944).
[3] W. B. MORS and O. RIBEIRO, *J. Org. Chem.* **22**, 978 (1957).
[4] R. L. WAIN, *Proc. Symposium on Potentials in Crop Protection*, New York State Agric. Exp. Station, Cornell University, Geneva, 26 (1969).
[5] M. G. CAVALIE, *Compt. Rend.* **238** (1), 689 (1964); *Chem. Abs.* **60**, 12384 (1964).
[6] CH. GREBUS, M. AUBERT-MINAGLOU and A. BORIS, *Trav. Soc. Pharm. Montpellier* **22** (1), 65 (1962); *Chem. Abs.* **59**, 920 (1963).

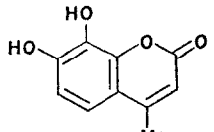
at 500 ppm. It was inactive against *B. subtilis*. In contrast to the latter observation, Fujikawa *et al.*[7] earlier reported that daphnetin is active against *B. subtilis*, as well as *Micrococcus pyogenes* and *E. coli*, at concentrations of only 100 ppm. In 1963, Senig[8] noted that extracts of leaves of *Acer platanoides* and *Aesculus hippocastanum* showed pronounced inhibition of growth of the cellulose-decomposing bacteria, *Sporocytophaga myxococcoides* and *Polyanguim cellulosum*. The most active constituent in the leaf extracts was shown to be aesculetin, which strongly inhibited growth of these bacteria at concentrations of 300–500 ppm. Scopoletin, isolated from potato tubers, was recently reported[9] to effectively inhibit sporulation and hyphal growth of *Fusarium solani*. The action of daphnetin and aesculetin derivatives on fungal growth does not appear to have been extensively examined, although it has been reported that these compounds have no effect on the growth of *Saccharomyces cerevisiae* and other yeasts.[10,11]

RESULTS AND DISCUSSION

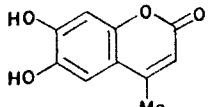
The inhibitory effects of daphnetin, aesculetin and related coumarins at concentrations of 500 ppm on the growth of twenty-two species of bacteria, yeasts and molds, have now been measured by Lederberg's[12] replica plating procedure. The effect of methylation, allylation, and acylation on the antibacterial and antifungal properties of the phenolic



(IV)



(V)



(VI)

IVa, R = H ; b, R = acetyl.

coumarins is shown in Table 1. In general accord with earlier[6,7] observations, daphnetin (Ia) completely inhibited the growth of the four Gram-positive and the five Gram-negative bacteria. It had no effect on the growth of yeasts and molds. Alkylation of daphnetin to give the 7-*O*-methyl derivative (IVa), the dimethyl derivative (Ib) or the diallyl derivative (Ic) resulted in the loss of its antibacterial activity. In contrast to daphnetin, however, these ethers showed fungistatic activity against some species of yeasts and molds; e.g. the monomethyl derivative (IVa) completely inhibited the growth of the yeast, *Candida tropicalis*, and of the molds, *Botrytis cinerea* and *Byssochlamys fulva*. Unlike alkylation, acetylation of daphnetin did not result in loss of antibacterial activity. The diacetate (Id) inhibited growth of all nine bacteria and, in addition, had a slight, inhibitory effect on the yeasts *Zygosaccharomyces japonicus* and *Candida tropicalis*. 4-Methyldaphnetin (V) had no effect on the growth of either bacteria or fungi

The antimicrobial activity of aesculetin (IIa) was similar to that of daphnetin. It strongly inhibited growth of Gram-positive bacteria and of two species (*Alcaligenes faecalis* and *Escherichia coli*) of Gram-negative bacteria, but had no effect on the growth of fungi.

[7] F. FUJIKAWA, K. NAKAJIMA, O. WADAI, M. TORII, S. NAKAZAWA, T. OMATSU and T. TOYODA, *J. Pharm. Soc., Japan* **73,** 250 (1953).
[8] E. SENIG, *Zentr. Bakteriol. Parasitenk*, Abt. II, **117** (1), 13 (1963), *Chem. Abs.* **60,** 8393 (1964).
[9] O. L. OZERETSKOVSKAYA, N. I. VASYUKOVA, M. A. DAVYDOVA and A. N. BAKH, *Prikl. Biokhim. Mikrobiol.* **4** (6), 698 (1968); *Chem. Abs.* **70,** 85 (1969).
[10] J. A. DE GREEF and C. F. VAN SUMERE, *Arch. Intern. Physiol. Biochim.* **74,** 512 (1966).
[11] V. DADÁK, *Pharmazie* **22,** 47 (1967).
[12] J. LEDERBERG and E. M. LEDERBERG, *J. Bacteriol.* **63,** 399 (1952).

TABLE 1. EFFECT OF COUMARINS ON THE GROWTH OF BACTERIA AND FUNGI

Coumarin	Bacteria									Fungi											
	Bacillus cereus 2006	*Sarcina lutea*	*Staphylococcus aureus* SG8A	*Streptococcus lactis*	*Alcaligenes faecalis* B170	*Escherichia coli* ML30	*Pseudomonas aeruginosa* 111	*Salmonella typhimurium* TmI	*Serratia marcescens*	*Zygosaccharomyces japonicus* C124	*Candida tropicalis* C147	*Pichia chodati* var. *fermentans* C238	*Hansenula anomala*	*Saccharomyces cerevisiae* var. *ellipsoideus*	*Torula utilis* NRRL Y660	*Aspergillus flavus* NRRL 3145	*Aspergillus niger* A-7705	*Penicillium chrysogenum* 52	*Rhizopus senti* NRRL 2868	*Botrytis cinerea* NRRL 3492	*Byssochlamys fulva* NRRL 3493	*Alternaria* spp.
Ia	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Ib	−	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−	−	−	−	−
Ic	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	+	±		
Id	+	±	±	±	+	+	+	+	±	±	±	−	−	−	−	−	−	−	−	−	−	
IVa	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−		+	+	−
IVb	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−		+	+	±
V	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
IIa	+	+	+	±	+	+		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
IIb	+	−	−	−	−	−		−	−	±	−	−	−	±	−	±	±	±	±	+	+	+
IIc*	+	±	±	±	+	−		−	−	+	+	±	±	+	+	±	±	±	±		±	+
IIIa	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+			+	
IIIb	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−				
IIIc	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−				
VI	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−
VIIa	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
VIIb	±	−	±	±	±	±		±	±	+	+	+	±	±	+	+	+	+	+		+	+
VIIc	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
VIId	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
VIIe	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
VIII	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+		+	+
IXa	−	−	−	−	−	±	−	−	±	±	±	−	−	−	−	−	−	±	−		±	
IXb	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−			+	
IXc	−	−	−	−	−	−	−	−	−	+	+	±	±	±	±	+	+	+	+	+	±	
IXd	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	
IXe	−	−	−	±	−	±	−	−	−	+	+	−	−	−	−	−	−	+	−	+	+	+
IXf	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	±	±	+	−	+	+	+

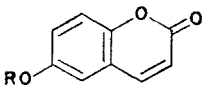
* Measured after 48 hr. The results are expressed on the basis: + = complete growth inhibition. ± = not completely effective; faint growth occurs. − = ineffective; growth occurs.

Methylation of aesculetin to give 6,7-dimethoxycoumarin (IIb), recently identified[13] as a major constituent of *Pterocaulon sphacelatum*, resulted in the loss of antibacterial activity and the development of fungistatic activity, particularly against molds. Like aesculetin, 6,7-diacetoxycoumarin exhibited a bacteriostatic effect. In contrast to aesculetin, however, the diacetate (IIc) was also fungistatic, and it inhibited growth of all yeasts and molds

[13] S. R. JOHNS, J. A. LAMBERTON, J. R. PRICE and A. A. SIOUMIS, *Austral. J. Chem.* **21**, 3079 (1968).

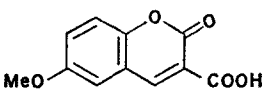
examined for a period of 48 hr. Scopoletin (IIIa) was ineffective against bacteria, although it did inhibit growth of the molds, *Penicillium chrysogenum* and *Byssochlamys fulva*. Substitution of aesculetin at position four to give 4-methylaesculetin (VI), and of scopoletin at position four to give the carboxylic acid derivatives (IIIb) and (IIIc) resulted in complete loss of antimicrobial activity.

In the preceding paper it was reported that the inhibitory action of umbelliferone is enhanced by *O*-methylation or acetylation. It was of interest to determine whether the antimicrobial properties of the isomeric 6- and 8-monohydroxycoumarins are similarly affected. Neither of these coumarins have yet been found in plants, and their effects on microbial growth do not appear to have been examined previously. Like umbelliferone, 6-hydroxycoumarin (VIIa) was completely ineffective against all bacteria and fungi with the exception of *Botrytis cinerea*. 6-Methoxycoumarin (VIIb), however, produced an inhibition in the growth of all Gram-positive and Gram-negative bacteria, three of the six yeasts, and all of the seven molds examined. The effect of methylation in this case,

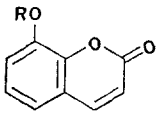


(VII)

VII a, R = H;
b, R = methyl;
c, R = acetyl;
d, R = allyl;
e, R = benzoyl.



(VIII)



(IX)

IXa, R = H;
b, R = methyl;
c, R = allyl,
d, R = benzoyl;
e, R = acetyl,
f, R = propionyl.

therefore, is even more pronounced than with umbelliferone. 6-Methoxycoumarin 3-carboyxlic acid (VIII) had no effect on bacteria; however, it completely inhibited growth of twelve species of fungi. The acetyl (VIIc), allyl (VIId) and benzoyl (VIIe) derivatives of 6-hydroxycoumarin were generally as inactive as the parent compound.

8-Hydroxycoumarin (IXa) was largely ineffective against bacteria and fungi, although it did produce slight inhibition of the growth of a few species. Methylation of 8-hydroxycoumarin, in contrast to umbelliferone and 6-hydroxycoumarin, resulted in complete loss of antimicrobial activity. 8-Acetoxy- (IXe), 8-propionyloxy- (IXf) and 8-allyloxycoumarin (IXe) were ineffective against bacteria. However, all of these derivatives were quite strongly fungistatic against a variety of yeasts and molds.

Approximate minimal inhibitory concentrations against bacteria and fungi were determined for dapnetin (Ia), aesculetin (IIa), and the active methyl and acetyl derivatives (Ia, IIc, VIIb). All were effective only at concentrations between 250–500 ppm, and these effective inhibitory concentrations were virtually unaffected by changes in pH in the range pH 4–pH 7.

EXPERIMENTAL

The coumarins used in this investigation were synthesized by standard procedures. Purity was checked by elemental analyses and NMR spectra. Microbiological assay, was carried out by Lederberg's replica plating technique as described in the previous paper in this series.[14]

[14] L. JURD, A. D. KING, JR. and K. MIHARA, *Phytochem.* **10**, 2965 (1971)