Antimicrobial Properties of Natural Phenols and Related Compounds II: Cinnamylated Phenols and Their Hydrogenation Products

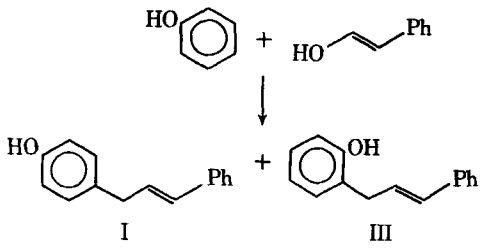
L. JURD, K. L. STEVENS, A. D. KING, Jr., and K. MIHARA*

Abstract □ A variety of nuclear alkylated *C*-cinnamylphenols were synthesized and tested for antimicrobial activity. These compounds are particularly effective against Gram-positive bacteria.

Keyphrases □ Cinnamylphenols and hydrogenation products—synthesized as possible antimicrobial agents, screened □ Antimicrobial agents, potential—synthesis of cinnamylphenols and hydrogenation products, pharmacological screening □ Phenols, cinnamylated and hydrogenation products—synthesized as possible antimicrobial agents, screened

It was recently reported (1) that obtusastyrene, a phenolic constituent of *Dalbergia sisoo* (2), is a potent, natural fungicide, the presence of it and a number of related compounds possibly being responsible for the high resistance of the heartwood of this species to fungal attack. Obtusastyrene completely inhibits the growth *in vitro* of a variety of Gram-positive bacteria, yeasts, and molds; in this respect, its activity compares favorably with that of a number of synthetic phenolic preservatives in current use.

The structure of obtusastyrene was established as *trans*-4-cinnamylphenol. Since, with the exception of obtusastyrene, the antibacterial and antifungal properties of *C*-cinnamylated phenols had not been examined previously, a variety of nuclear alkylated analogs of obtusastyrene was synthesized and screened *in vitro* for their effects on the growth of microorganisms.



Scheme I

MATERIALS AND METHODS

Cinnamylation of Phenols: General Procedure—Obtusastyrene (I) is easily synthesized in quantity by the acid-catalyzed condensation of cinnamyl alcohol with phenol in aqueous solutions (3, 4). The isomeric 2-cinnamylphenol (III) is formed as a minor product in this reaction (Scheme I).

o-, *m*-, and *p*-Cresol, thymol, carvacrol, and other 4-alkylphenols were cinnamylated under similar conditions to yield the condensation products listed in Table I. Separation of pure products was effected by distillation and, where applicable, by repeated crystallization. Identity of all products was confirmed by elemental analyses and by determination of their NMR spectra. In this connection, it is noteworthy that the NMR spectra of the 1,3-substituted propene portion of the cinnamylated phenols may be used to estab-

Table I—Cinnamylated Phenols and Their Hydrogenation Products

Reactant Phenol	Compound	Product: Identity	Molecular Formula	Boiling Point	Melting Point
Phenol	I	4-Cinnamylphenol (obtusastyrene)	$C_{15}H_{14}O$	169°/0.4 mm.	64°
	II	Dihydro-4-cinnamylphenol	$C_{15}H_{16}O$	—	63–64°
	III	2-Cinnamylphenol	—	—	57°
	IV	Dihydro-2-cinnamylphenol	—	—	—
*p*Cresol	V	2-Cinnamyl-4-methylphenol	$C_{16}H_{16}O$	170°/0.4 mm.	57°
	VI	Dihydro-2-cinnamyl-4-methylphenol	$C_{16}H_{18}O$	155°/1.0 mm.	—
o-Cresol	VII	4-Cinnamyl-2-methylphenol	$C_{16}H_{16}O$	—	71°
	VIII	Dihydro-4-cinnamyl-2-methylphenol	$C_{16}H_{18}O$	165°/0.2 mm.	—
	IX	2-Cinnamyl-6-methylphenol	$C_{16}H_{16}O$	147–148°/0.2 mm.	—
m-Cresol	X	4-Cinnamyl-3-methylphenol + 2-cinnamyl-5-methylphenol	$C_{16}H_{16}O$	150–170°/0.05 mm.	—
4-Ethylphenol	XI	2-Cinnamyl-4-ethylphenol	$C_{17}H_{18}O$	—	70–70.5°
	XII	Dihydro-2-cinnamyl-4-ethylphenol	$C_{17}H_{20}O$	—	29–30°
4-*n*-Propylphenol	XIII	2-Cinnamyl-4-*n*-propylphenol	$C_{18}H_{20}O$	—	38–40°
	XIV	Dihydro-2-cinnamyl-4-*n*-propylphenol	$C_{18}H_{22}O$	—	—
4-Isopropylphenol	XV	2-Cinnamyl-4-isopropylphenol	$C_{18}H_{20}O$	—	59–62°
	XVI	Dihydro-2-cinnamyl-4-isopropylphenol	$C_{18}H_{22}O$	—	—
2,4-Dimethylphenol	XVII	2-Cinnamyl-4,6-dimethylphenol	$C_{17}H_{18}O$	187°/1.0 mm.	—
Thymol	XVIII	4-Cinnamyl-3-methyl-6-isopropylphenol	$C_{19}H_{22}O$	175°/0.03 mm.	—
	XIX	Dihydro-4-cinnamyl-3-methyl-6-isopropylphenol	$C_{19}H_{24}O$	170°/0.05 mm.	—
	XX	2-Cinnamyl-3-methyl-6-isopropylphenol	$C_{19}H_{22}O$	175°/0.03 mm.	—
Carvacrol	XXI	4-Cinnamyl-2-methyl-5-isopropylphenol	$C_{19}H_{22}O$	185°/0.05 mm.	—
4-Methoxyphenol	XXII	2-Cinnamyl-4-methoxyphenol	$C_{16}H_{16}O_2$	213°/2.4 mm.	90°
	XXIII	Dihydro-2-cinnamyl-4-methoxyphenol	$C_{16}H_{18}O_2$	—	66°

Table II—Effects of Cinnamylated Phenols and Their Dihydro Derivatives on Growth of Bacteria and Fungi[a]

	Bacteria									Fungi													
Compound	*Bacillus cereus* 2006	*Sarcina lutea*	*Staphylococcus aureus* SG8A	*Streptococcus lactis*	*Acaligenes faecalis* B170	*Escherichia coli* ML30	*Pseudomonas aeruginosa* III	*Salmonella typhimurium* Tm1	*Serratia marcescens*	*Zygosaccharomyces japonicus* C124	*Candida tropicalis* C147	*Pichia chodatii*/var. fermentans C238	*Hansenula anomala*	*Saccharomyces cerevisiae*/var. ellipsoideus Y44	*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* sp.
I	25	25	25	25	50–100	50–100	–	–	–	12	12	25	50	25	50	100	50	50	6	25–50	25	25
II	25	25–50	25–50	25	200	200	200	200	200	25	25	50	50	25	25	200	50–100	25	50	12–25	50	25
III	12	25	25	25	100	100	–	–	–	12	12–25	50	50	25	50	100	100	25–50	50	12–25	25	25
IV	12	25	25	12	–	–	–	–	–	12	12	50	25	12	25	50	50	25	25	12	25	25
V	12	12	12	12	–	–	–	–	–	12	25	>200	50	25	>200	>200	>200	100	>200	25	25	100
VI	12	12	12	12	–	–	–	–	–	12	12	–	25		12	>200	>200	200	100	>200	>200	100
VII	12	12	12	12	–	–	–	–	–	12	25	200	100	25	100	>200	>200	50	50	100	25	50–100
VIII	12	12	12	12	–	–	–	–	–	12	12	>200	100	12	>200	200	>200	25	50	25	25–50	25–50
IX	12	12	12	12	–	–	–	–	–	25	25	–	–	25	>200	>200	>200	>200	>200	200	>200	>200
X	12	25	25	12	–	–	–	–	–	12	12	200	–	>200	200	200	100	50	50	100	100	100
XI	12	12	12	12	–	–	–	–	–	12	12	–	–		–	–	>200	200	100	200	200	200
XII	12	12	12	12	–	–	–	–	–	12–25	12–25	–	–	50	100	–	–	>200	–	100–200	>200	>200
XIII	12	12	12	12	–	–	–	–	–	>200	50–100	–	–	–	–	>200	>200	–	>200	–	–	–
XIV	25	12	12	12	–	–	–	–	–	200	200	–	–	–	–	–	–	–	–	–	–	–
XV	12	12	12	12	–	–	–	–	–	25–50		–	–	–	–	–	–	–	200	200	–	–
XVI	12	25	12–25	12	–	–	–	–	–	50	12	–	–	–	–	>200	>200	–	>200	100	–	–
XVII	12		12	12	–	–	–	–	–	12	12	–	–	–	–	–	–	–	–	–	–	–
XVIII	±	–	+	+	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	+	–	±
XIX	+	+	+		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XX	±	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	±	–	±	+	+
XXI	+	+	+	++	–	–	–	–	–													
XXII	25	25	25	25	–	–	–	–	–	25	50	>200	100	50	>200	–	–	>200	–	>200	50	100–200
XXIII	13	12	25–50	25	–	–	–	–	–	25	25	–	50	25	200	–	–	>200	100	200	200	200
															–	200		–	–			

[a] Numbers refer to approximate minimal inhibitory concentrations in micrograms/milliliter. >200 = growth not inhibited with 200 mg./ml. (highest concentration tested). + = complete inhibition of growth with 500 mcg./ml. These compounds were not tested at lower concentrations. – = no growth inhibition with 500 mcg./ml. ± = slight growth with 500 mcg./ml.

lish the location of the cinnamyl group relative to the phenolic hydroxyl group. Thus, it was found that the methylene doublet (J = 5 Hz.) occurs in the region δ 3.40–3.44 when it is *para* to the hydroxyl (as in obtusastyrene, I) and in the region δ 3.46–3.57 when it is *ortho* to the hydroxyl (as in Compounds III and V). Only one exception to this generalization was noted—*viz.*, in Compound XXI the methylene doublet occurs at δ 3.50. The position of the low multiplet due to the vinyl proton adjacent to the allylic methylene group is also characteristic. The methine proton in those compounds in which the cinnamyl group is *para* to the hydroxyl occurs in the region δ 6.16–6.24, while in the *ortho*-cinnamyl phenols it occurs between δ 6.25 and 6.30.

The cinnamylation of *o*-cresol illustrates the general reaction procedure: a solution of *o*-cresol (108 g.) and cinnamyl alcohol (68 g.) in formic acid (200 ml.) and water (80 ml.) was heated on a steam bath for 30 min. Distillation of the oily product, which separated upon addition of water (800 ml.), gave a fraction, b.p. 163–168°/0.2 mm. (83 g.). This fraction was dissolved in ether (60 ml.) and slowly diluted at boiling with Skellysolve F (600 ml.). Colorless needles, m.p. 71°, separated on cooling. Recrystallized from ether–Skellysolve F, pure 4-cinnamyl-2-methylphenol (7) was obtained as colorless prismatic needles, m.p. 71° [lit. (5) m.p. 71–72°].

Anal.—Calc. for $C_{16}H_{16}O$: C, 85.7; H, 7.19. Found: C, 85.6; H, 7.27.

Chromatography of the ether–Skellysolve F filtrate showed the presence of residual 4-cinnamyl-2-methylphenol and a second minor reaction product. The ether–Skellysolve F solution was extracted with 10% aqueous KOH (3 × 100 ml.), which preferentially removes the 4-cinnamyl compound, until chromatography of the ether–Skellysolve F solution showed the presence of only the minor product. Evaporation and distillation of the solution then gave 2-cinnamyl-6-methylphenol (IX) as a colorless oil, b.p. 147–148°/0.2 mm. (4.1 g.).

Anal.—Calc. for $C_{16}H_{16}O$: C, 85.7; H, 7.19. Found: C, 85.4; H, 7.24.

Hydrogenation of Cinnamylphenols—The *trans*-cinnamylphenols (10 g.) in tetrahydrofuran (50 ml.) were converted into the saturated dihydro derivatives by hydrogenation at room temperature and approximately 30 lb. pressure in the presence of a 5% palladium–carbon catalyst. The dihydro derivatives were purified by distillation under reduced pressure or by crystallization.

Antimicrobial Bioassay—All compounds were initially tested at a concentration of 200 or 500 p.p.m. (w/v). Plates were prepared by adding a measured amount of the phenol 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 and age overnight. The plates were then inoculated with the test organisms. In the case of bacteria and yeasts, the inoculation was done by the Lederberg and Lederberg (6) replica plating technique, 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 or four molds per plate. The media used were: plate count agar (Difco), pH 7.0, for bacteria; and potato dextrose agar (Difco), pH 5.6, for yeasts and molds. Inoculated control plates were also prepared with media containing the largest amount of acetone (0.80 ml.) used in the preparation of the test plates. We have determined that acetone has a minimal influence on the growth of organisms in this procedure. The plates were incubated at 28° for 1–5 days and evaluated by comparison with the controls.

RESULTS AND DISCUSSION

The *trans*-cinnamylphenols and their dihydro derivatives were screened initially at the high concentration (500 mcg./ml.) for activity against 22 representative microorganisms by the Lederberg and Lederberg (6) replica plating technique. The minimal concentrations of active compounds required for complete inhibition of microbial growth were then determined (Table II). A number of di-*C*-cinnamylphenols, as well as various derivatives (acetates, benzoates, and methyl ethers) of the mono-*C*-cinnamylphenols, showed no activity at high concentrations and are not included in the table.

As indicated in Table II, the antimicrobial activities of 2-cinnamylphenol (III) and its dihydro derivative (IV) are comparable with those of obtusastyrene (I) and dihydroobtusastyrene (II). All four compounds strongly inhibit the growth of Gram-positive bacteria (*Bacillus cereus*, *Sarcina lutea*, *Staphylococcus aureus*, and *Streptococcus lactis*) at concentrations of about 25 mcg./ml. and of 11 species of yeasts and molds at concentrations of 12–50 mcg./ml. Higher concentrations (50–100 mcg./ml.) were required for inhibition of growth of *Aspergillus* species. These compounds are generally ineffective against Gram-negative bacteria, although at higher concentrations (200 mcg./ml.) dihydroobtusastyrene (II) completely inhibits the growth of all five species of Gram-negative bacteria examined.

In considering the effect of nuclear alkylation on the antimicrobial properties of the cinnamylphenols (I and III) and their dihydro derivatives, it is apparent from Table II that the introduction of methyl, ethyl, and propyl groups (Compounds V–XVII) enhances the activity of these compounds against Gram-positive bacteria (growth inhibition of all four species occurs with 12 mcg./ml. or less). However, with the exception of their inhibitory effect on the yeasts *Zygosaccharomyces japonicus* and *Candida tropicalis*, nuclear alkylation markedly lowers their activity against fungi.

Studies in progress indicate that the action of the cinnamylcresols (Compounds V and VII) is bactericidal, and it is rapidly exerted against *S. aureus* and other Gram-positive organisms.

REFERENCES

(1) L. Jurd, A. D. King, Jr., K. Mihara, and W. L. Stanley, *Appl. Microbiol.*, **21**, 507(1971).

(2) M. Gregson, K. Kurosawa, W. D. Ollis, G. T. Redman, R. J. Roberts, I. O. Sutherland, A. B. DeOliveira, W. B. Eyton, and O. R. Gottlieb, *Chem. Commun.*, 2(22), 1390(1968).

(3) L. Jurd, *Tetrahedron Lett.*, **33**, 2863(1969).

(4) S. Mageswaran, W. D. Ollis, R. J. Roberts, and I. O. Sutherland, *ibid.*, **34**, 2897(1969).

(5) O. Mumm and F. Möller, *Chem. Ber.*, **70B**, 2214(1937).

(6) J. Lederberg and E. M. Lederberg, *J. Bacteriol.*, **63**, 399 (1952).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 7, 1971, from the *Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, CA 94710*

Accepted for publication August 3, 1971.

* Present address: Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Berkeley, CA 94710