Antimicrobial Properties of Substituted Salicylaldehydes and Related Compounds

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A systematic survey of the antimicrobial properties of substituted salicylaldehydes and some related aromatic aldehydes is reported. A total of 23 different compounds, each at four different concentrations, were studied using a panel of seven microbes (*Aspergillus niger*, *Bacillus cereus*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* and *Staphylococcus epidermidis*) and employing the paper disc agar diffusion method. Several aldehydes, most notably halogenated, nitro-substituted and hydroxylated salicylaldehydes, displayed highly potent activity against the microbes studied, giving inhibitory zones up to 49 mm in diameter (paper disc diameter 6 mm), while unsubstituted benzaldehyde and salicylaldehyde had minimal activity. Further, 4,6-dimethoxysalicylaldehyde had considerable activity against *C. albicans* and slight activity against *S. cerevisiae*, while displaying minimal activity against bacteria. Also two aromatic dialdehydes had interesting activity. In general, *P. aeruginosa* was the least sensitive microbe, a result that is in line with observations from a large screening project, in which this microbe was the one against which the least number of active substances was found. Interestingly, the structure-activity relationships of the aldehydes studied were clearly different for different microbes. Many of the aldehydes tested had such high antimicrobial activity that they are noteworthy candidates for practical applications as well as interesting lead compounds for the development of novel antimicrobial drugs and disinfectants. The structure-activity relationships are discussed in detail. For high activity, substituents are required in benzaldehyde as well as in its 2-hydroxy derivative salicylaldehyde. One hydroxy group alone (at the 2-position) is not enough, but further hydroxylation may produce high activity. The effects of substituents are in some cases dramatic. Halogenation, hydroxylation and nitro substitution may produce highly active compounds, but the effects are not easily predicted nor can they be extrapolated from one microbe to another.

Key words: Antibacterial Agents, Antifungal Agents, Substituent Effects

Introduction

There are sporadic reports on the antimicrobial activity of certain substituted salicylaldehydes (ring-substituted 2-hydroxybenzaldehydes), a few of which are known to have antimicrobial activity against certain bacteria or fungi (Bougault *et al.*, 1949; Burton *et al.*, 1964, 1965; Clarke *et al.*, 1963; Cronenberger *et al.*, 1968a, b, 1969; Rehn *et al.*, 1981; Taillandier and Pera, 1991). The ultimate mechanism underlying the antimicrobial activity is not known, and in most studies, the number of compounds tested has been quite limited or only one microbial species has been studied. Little appears to be known of the effects of substituents of the ring, and at present, the mechanism of action of the aldehydes is at best speculative. One possibility is that the activity is based on the formation of Schiff bases with important amino groups of microbial cells.

Because of the above reasons, a further characterization of the antimicrobial properties of substituted salicylaldehydes is of great interest. This is even more so, since in recent times, bacteria resistant to commonly used antibiotics have caused much alarm. For example, vancomycin-resistant

enterococci (VRE) and methicillin-resistant staphylococci, most notably methicillin-resistant *Staphylococcus aureus* (MRSA), have become serious clinical problems world-wide (Bozdogan *et al.*, 2003; Centers for Disease Control and Prevention, 2002; Cetinkaya *et al.*, 2000; Cunha, 2005; Hanaki *et al.*, 2005; Hsueh *et al.*, 2005; Weigelt *et al.*, 2004). The clinically useful antibiotic arsenal available against these bacteria is limited and new resistant mutants are emerging that still worsen the situation. This has prompted an intensive search for new active agents. As part of a large project to invent novel antimicrobial agents, we have synthesized *e. g.* certain hydrazone-type and other derivatives of substituted salicylaldehydes and studied their antimicrobial properties (Pelttari *et al.*, 2007; unpublished results). In connection with those studies, we have performed a systematic study of the antimicrobial properties of a large number of ring-substituted salicylaldehydes using a panel of seven microbial species (including bacteria, yeasts and one mold), the results of which are reported here. Many of the aldehydes tested were found to have highly potent antimicrobial activity, making these substances noteworthy candidates for practical applications as well as interesting lead compounds for the development of novel antimicrobial compounds.

Experimental

Compounds tested

The structures of the compounds studied are shown in Fig. 1. The aldehydes tested were obtained from E. Merck and Schuchardt (Darmstadt, Germany), Aldrich-Chemie/Aldrich Chemical Company and EGA-Chemie (Steinheim, Germany), Fluka AG (Buchs, Switzerland) and TCI (Tokyo, Japan). These compounds were used as such without further purification. Benzaldehyde was distilled before use, and an undistilled lot was used for comparison.

3,5-Difluorosalicylaldehyde was synthesized in our laboratory. The preparation was based on Duff's reaction wherein the appropriate phenol is regiospecifically formylated by two molar equivalents of hexamethylenetetramine (*i. e.*, urotropine) in trifluoroacetic acid. The crude product of the aldehyde was synthesized according to Weidner-Wells and Fraga-Spano (1996) starting from 99 mmol of 2,4-difluorophenol, but was purified further (instead of oxidation to 3,5-difluorosalicylic acid) as follows: the concentrated crude product (ca. 50 ml) was mixed with 100 ml of dichloromethane and filtered. The filtrate was washed with water in a separatory funnel, dried with a small amount of sodium sulfate and concentrated. The residue was recrystallized from 7 ml of 50% methanol in water; yield 36%; melting point 85–86 °C. The solid by-product that was filtered off did not melt below 350 °C.

Attempts to synthesize other di- or trifluorosalicylaldehydes by a similar method (Duff's reaction) were unsuccessful as expected because of the deactivating effects of the fluorine substituents on *ortho* and *para* positions.

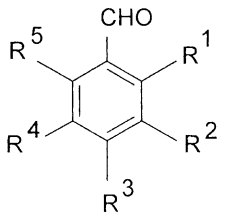
Microbiology

The compounds were tested against *Aspergillus niger*, *Bacillus cereus*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* and *Staphylococcus epidermidis*. The microbial strains, their origins and preservation, culture media and conditions and the experimental procedures employed have been previously described (Pelttari *et al.*, 2002). In brief, for testing of antimicrobial activities, single colonies of the microbes were taken from agar plates and grown aerobically (at 30 or 37 °C, depending on the microbe) in several 5 ml aliquots of the appropriate liquid medium and using orbital shaking (120 rpm). Liquid cultures from overnight cultivations (5 ml each) were centrifuged, the pellets were washed, recentrifuged and resuspended (volume 400 μl), and 200 μl of this suspension was inoculated onto each plate (diameter 14 cm, approximately 50 ml of agar). Paper discs (diameter 6 mm) were put onto the plates, and 10 μl of a test solution were pipetted onto each disc. All compounds studied were dissolved in dimethyl sulfoxide (DMSO).

Results and Discussion

General remarks

A total of 23 different aldehydes were tested for their ability to inhibit microbial growth using seven different microbes as test organisms, namely four bacterial species (*B. cereus*, *E. coli*, *P. aeruginosa* and *S. epidermidis*) and two yeasts (*C. albicans* and *S. cerevisiae*) as well as one mold (*A. niger*). The structures of the compounds are shown in Fig. 1. In the case of compounds **3** and **19**, lots from two different suppliers were employed. The



1: $R^1 = R^2 = R^3 = R^4 = R^5 = H$
2: $R^1 = OH$, $R^2 = R^3 = R^4 = R^5 = H$
3: $R^1 = R^2 = OH$, $R^3 = R^4 = R^5 = H$
4: $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = R^5 = H$
5: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = OH$, $R^5 = H$
6: $R^1 = H$, $R^2 = R^3 = OH$, $R^4 = R^5 = H$
7: $R^1 = R^2 = R^3 = OH$, $R^4 = R^5 = H$
8: $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = H$, $R^5 = OH$
9: $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = H$, $R^5 = CH_3$
10: $R^1 = OH$, $R^2 = H$, $R^3 = OCH_3$, $R^4 = H$, $R^5 = OCH_3$
11: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = OCF_3$, $R^5 = H$
12: $R^1 = OH$, $R^2 = F$, $R^3 = R^4 = R^5 = H$
13: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = Cl$, $R^5 = H$
14: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = Br$, $R^5 = H$
15: $R^1 = OH$, $R^2 = F$, $R^3 = H$, $R^4 = F$, $R^5 = H$
16: $R^1 = OH$, $R^2 = Cl$, $R^3 = H$, $R^4 = Cl$, $R^5 = H$
17: $R^1 = OH$, $R^2 = Br$, $R^3 = H$, $R^4 = Br$, $R^5 = H$
18: $R^1 = OH$, $R^2 = I$, $R^3 = H$, $R^4 = I$, $R^5 = H$
19: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = NO_2$, $R^5 = H$
20: $R^1 = OH$, $R^2 = NO_2$, $R^3 = H$, $R^4 = NO_2$, $R^5 = H$
21: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = CHO$, $R^5 = H$
22: $R^1 = CHO$, $R^2 = R^3 = R^4 = R^5 = H$
23: $R^1 = R^2 = H$, $R^3 = N(CH_3)_2$, $R^4 = R^5 = H$

Fig. 1. The structures of the aldehydes studied.

aldehydes tested include salicylaldehyde [*i.e.*, 2-hydroxybenzaldehyde (**2**)] and ring-substituted analogues thereof as well as the "parent compound" of all aromatic aldehydes, benzaldehyde (**1**), and a few other aromatic aldehydes. The results obtained are shown in Table I. As is evident from Table I, several of the aldehydes tested had highly potent antimicrobial activity, producing inhibitory zones up to 49 mm in diameter. Every aldehyde had at least some activity against one or more microbial strains.

Unsubstituted benzaldehyde and salicylaldehyde had minimal activity. The effect of substituents was dramatic, especially in the case of salicylaldehyde, whose activity was greatly increased by halogenation, nitro substitution and hydroxylation.

Each microbial strain was inhibited by at least one of the aldehydes tested. In general, the least sensitive microbe was *P. aeruginosa*. This result is in line with our observations from a large screening project, in which this microbe was the one against which the least number of active substances was found. Interestingly, the structure-activity relationships of the aldehydes studied varied for different microbes.

Effects of halogen substituents on antimicrobial activity

A total of seven halogenated salicylaldehydes, including 3- and 5-monohalogenated as well as 3,5-dihalogenated ones, were available for this study. The structure-activity relationships observed for these compounds are illustrated graphically in Fig. 2.

Three monohalogenated salicylaldehydes [3-fluorosalicylaldehyde (**15**), 5-chlorosalicylaldehyde (**13**) and 5-bromosalicylaldehyde (**14**)] were available for testing. Among them, the 3-fluoro congener had fairly high activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa* as well as against the Gram-positive *B. cereus*, while being inactive against the Gram-positive *S. epidermidis* as well as the mold *A. niger* and practically inactive against the yeasts *C. albicans* and *S. cerevisiae.*

5-Bromosalicylaldehyde was in most cases highly active, being drastically more active than 5-chlorosalicylaldehyde when comparing solutions with the same mg/ml concentration, and even more active if molar concentrations are considered in the comparison; this result is difficult to explain. In the case of *S. cerevisiae*, the activity of the 5-bromo congener (although higher than that of the 5-chloro congener) was quite low. The higher activity of the bromo compound is in line with the results of Cronenberger *et al.* (1969) who studied the effects of aldehydes on *S. cerevisiae.*

In the case of *P. aeruginosa* and *A. niger*, both of the 5-halogenated congeners had fairly low (and, as compared to each other, roughly equal) activity, and in contrast to the other microbes, were more active than the 3,5-dihalogenated salicylaldehydes that usually had clearly superior activity. In the case of *E. coli*, the 3,5-dichloro congener **16** was much more active than the 5-chloro one **13**, while the 3,5-dibromo analogue **17** was less active than the highly effective 5-bromo one **14**.

In the case of 3,5-dihalogenated salicylaldehydes, highest activity was usually displayed by the dichloro congener. It is tempting to speculate that the increase of activity ongoing from the difluoro compound to the dichloro one is due to a change in the inductive effect of the substituents. The de-

Table I. The results of the growth inhibition tests.

Compound	Concentration [μM][b]	Concentration [mg/ml][b]	Mean diameter of inhibitory zone [mm][a]						
			B. cereus	*E. coli*	*P. aeruginosa*	*S. epidermidis*	*C. albicans*	*S. cerevisiae*	*A. niger*
1[c]	377	40	7	7	6	7	6	7	6
	184	20	6	6	6	6	6	7	6
	94	10	6	6	6	6	6	6	6
	47	5	6	6	6	6	6	6	6
1[d]	377	40	7	7	7	7	6	7	6
	184	20	6	6	7	6	6	6	6
	94	10	6	6	7	6	6	6	6
	47	5	6	6	6	6	6	6	6
2	328	40	7	7	6	7	7	8	6
	163	20	7	7	6	7	7	7	6
	82	10	7	6	6	6	6	7	6
	41	5	6	6	6	7	6	7	6
3[e]	290	40	25	21	13	21	23	29	ND
	145	20	11	16	11	14	30	30	ND
	72	10	8	9	9	10	23	28	ND
	36	5	6	6	6	6	23	24	ND
3[f]	290	40	29	23	15	26	40	ND	ND
	145	20	23	20	14	23	30	ND	ND
	72	10	15	15	12	21	25	ND	ND
	36	5	14	8	7	15	21	ND	ND
4	290	40	17	17	12	18	24	12	10
	145	20	11	16	10	10	17	8	7
	72	10	6	11	8	8	11	7	6
	36	5	6	7	6	6	6	6	6
5	290	40	19	17	16	16	19	6	11
	145	20	10	13	12	12	14	7	7
	72	10	7	7	9	10	8	6	6
	36	5	6	8	6	8	6	6	6
6	290	40	6	11	13	6	6	6	6
	145	20	6	8	11	6	6	6	6
	72	10	6	7	13	6	6	6	6
	36	5	6	7	6	6	6	6	6
7	260	40	14	23	13	12	8	7	7
	130	20	9	16	11	10	6	7	6
	65	10	8	12	8	8	6	6	6
	32	5	6	8	7	6	6	7	6
8	260	40	12	15	9	13	6	6	ND
	130	20	8	8	7	10	6	6	ND
	65	10	7	7	6	6	6	6	ND
	32	5	7	6	6	6	6	6	ND
9	263	40	13	15	9	13	13	10	9
	131	20	9	10	7	9	9	8	7
	66	10	8	7	7	7	7	7	6
	33	5	7	6	6	6	6	6	6

crease of activity after the dichloro stage when going to the dibromo- and diiodoaldehydes must of course in part be due to the increasing molecular weight that results in lower molar concentrations when the same mg/ml concentration is employed in testing. In the case of agar diffusion experiments, the role of this phenomenon is usually difficult to determine quantitatively. In the present case, however, it was observed (see Table I) that 105 μM dichlorosalicylaldehyde was in most cases a more potent inhibitor of microbial growth than is a 107 μM solution of the corresponding diiodoaldehyde and approximately equally active with both 70 and 150 μM solutions of the dibromo congener. The decrease of activity when going from the dichloro congener to the diiodo one may in part also be due to the drastically increasing size of the substituents. Another possibility is that the

Table I continued.

Compound	Concentration [μM][b]	Concentration [mg/ml][b]	Mean diameter of inhibitory zone [mm][a]						
			B. cereus	*E. coli*	*P. aeruginosa*	*S. epidermidis*	*C. albicans*	*S. cerevisiae*	*A. niger*
10	220	40	7	8	6	8	20	11	10
	110	20	7	9	6	8	17	11	9
	55	10	8	7	6	7	13	7	8
	27	5	6	8	7	6	6	7	6
11	206	40	6	11	9	8	9	10	6
	103	20	6	8	8	7	8	8	6
	52	10	6	8	8	6	9	8	6
	26	5	6	7	7	6	8	8	6
12	286	40	21	20	17	6	8	6	6
	143	20	7	20	13	6	7	7	6
	71	10	6	8	6	6	7	7	6
	36	5	6	6	6	6	6	6	6
13	256	40	14	9	13	7	8	8	22
	128	20	11	8	13	6	8	8	10
	64	10	10	8	8	6	9	7	7
	32	5	8	9	7	6	7	7	6
14	199	40	26	32	12	22	25	12	19
	100	20	13	30	12	21	9	10	11
	50	10	12	17	10	7	7	7	7
	25	5	8	11	7	6	7	7	7
15	209	40	23	21	10	>30	7	23	ND
	105	20	15	11	9	ND	6	7	ND
	52	10	12	7	7	ND	6	7	ND
	26	50	11	6	6	ND	6	7	ND
16	209	40	29	34	8	33	32	32	16
	105	20	27	30	8	32	34	30	18
	52	10	22	27	7	28	23	21	12
	26	5	19	20	8	23	20	20	12
17	150	40	27	23	9	28	26	31	15
	71	20	27	23	9	28	26	31	15
	36	10	24	21	9	25	19	28	14
	18	5	20	16	9	22	16	18	12
18	107	40	23	16	9	33	15	12	10
	54	20	20	16	8	32	15	13	11
	27	10	20	21	8	32	15	11	10
	13	5	20	15	8	30	13	12	10
19[g]	239	40	18	21	6	32	27	29	16
	120	20	18	21	6	32	27	29	16
	60	10	10	17	6	32	20	24	13
	30	5	7	14	6	34	15	13	11
19[f]	239	40	20	24	8	49	25	26	20
	120	20	13	21	7	45	24	26	18
	60	10	10	18	6	44	21	21	15
	30	5	7	14	6	44	16	13	10

inductive effect of two bromines or iodines is too low for optimal activity.

Our results on dihalogenated salicylaldehydes are in contrast with those of Cronenberger *et al.* (1969), who studied the effects of aldehydes on *S. cerevisiae* and reported that the IC_{50} value of 3,5-diiodosalicylaldehyde (**18**) is lower than that of the dibromo congener, whose IC_{50} value in turn is lower than that of the dichloro congener. The reasons lying behind this discrepancy are not known but may perhaps relate to different experimental conditions and different strains of the microbe.

Effects of hydroxy substituents on antimicrobial activity

A large and highly interesting subgroup of the aldehydes studied is constituted by doubly or tri-

Table I continued.

Compound	Concentration [μM][b]	Concentration [mg/ml][b]	Mean diameter of inhibitory zone [mm][a]						
			B. cereus	*E. coli*	*P. aeruginosa*	*S. epidermidis*	*C. albicans*	*S. cerevisiae*	*A. niger*
20	189	40	14	20	8	8	6	7	6
	94	20	7	15	8	8	7	7	6
	47	10	7	8	7	7	6	7	6
	24	5	6	7	7	7	6	6	6
21	266	40	21	17	9	26	23	23	16
	133	20	14	17	8	12	20	24	14
	67	10	9	14	7	12	12	14	10
	33	5	7	12	6	9	9	7	7
22	298	40	9	13	16	13	17	24	11
	149	20	7	13	12	10	15	22	8
	75	10	7	10	9	10	13	17	7
	37	5	7	9	8	9	9	14	7
23	268	40	6	8	7	6	7	7	7
	134	20	6	8	7	6	6	7	6
	67	10	6	7	6	6	6	7	6
	34	5	6	7	6	6	6	6	6

[a] Diameter of filter paper disc was 6 mm. The diameters given include the disc diameter. For each concentration of each compound, four filter discs were employed, and for each disc the inhibitory zone diameter was measured in at least three directions using a standard ruler, whose smallest division was 1 mm. For each disc, the mean of the individual measurements was calculated and rounded to whole numbers. All four discs usually gave either the same inhibitory zone value or the differences were small. In the case of *A. niger*, the typical standard deviation (SD) was 0 mm. The control antibiotic amphotericin B (fungizone, 8 mg/ml) gave inhibitory zones of ca. 10–11 mm. In the case of *E. coli*, the SD ranged between 0 and 2 mm, and the control antibiotic (ampicillin sodium, 1 mg/ml) gave inhibitory zones around 20–22 mm. In the case of *S. epidermidis*, the typical SD was below 1 mm (maximum 4 mm in one case), and the control antibiotic (doxycycline, 20 mg/ml) gave inhibitory zones of ca. 21–24 mm. In the case of *B. cereus*, the SD was typically below 2 mm (often 0 mm), and doxycycline gave inhibitory zones of ca. 33 mm. In the case of *P. aeruginosa*, the SD was nearly always below 1 mm, and doxycycline gave inhibitory zones of ca. 18 mm. In the case of *C. albicans*, the SD was usually below 1 mm and amphotericin gave inhibitory zones of ca. 15–16 mm. In the case of *S. cerevisiae*, the SD was nearly always below 1 mm (often 0 mm), and amphotericin gave inhibitory zones of ca. 9 mm.

[b] Concentration of test substance in DMSO. 10 μl of this solution were pipetted onto each paper disc.

[c] Undistilled.

[d] Distilled.

[e] Product of Merck-Schuchardt.

[f] Product of Aldrich Chemical Company.

[g] Product of TCI.

ND, not determined.

ply hydroxylated benzaldehydes, many of which displayed potent antimicrobial activity. The structure-activity relationships observed for the doubly hydroxylated congeners are illustrated graphically in Fig. 3.

Among the doubly hydroxylated congeners, 2,3-dihydroxybenzaldehyde (**3**) was in most cases superior to its 2,4- and 2,5-analogues **4** and **5**. In the case of the bacteria *E. coli*, *S. epidermidis* and especially *B. cereus*, the 2,3-hydroxylated congener had so high activity that it may deserve consideration for practical antimicrobial use. Two lots of it were available for this study (one from Aldrich and one from Merck) and they gave largely similar results, except in the case of *B. cereus* and *S. epidermidis*, the Aldrich product giving at low concentrations somewhat larger inhibitory zones than the Merck product. These differences may well at least in part be due to experimental inaccuracy but the possibility cannot be excluded that either lot contained impurities, *e.g.* because the dihydroxy compound is probably easily oxidized to a quinone. The 3,4-dihydroxylated congener **6**, instead, had low or no activity against these bacteria. This result is interesting since the 3,4-substituted compound does not belong to salicylaldehydes, in contrast to the other dihydroxylated congeners studied. In the case of *P. aeruginosa*, all four di-

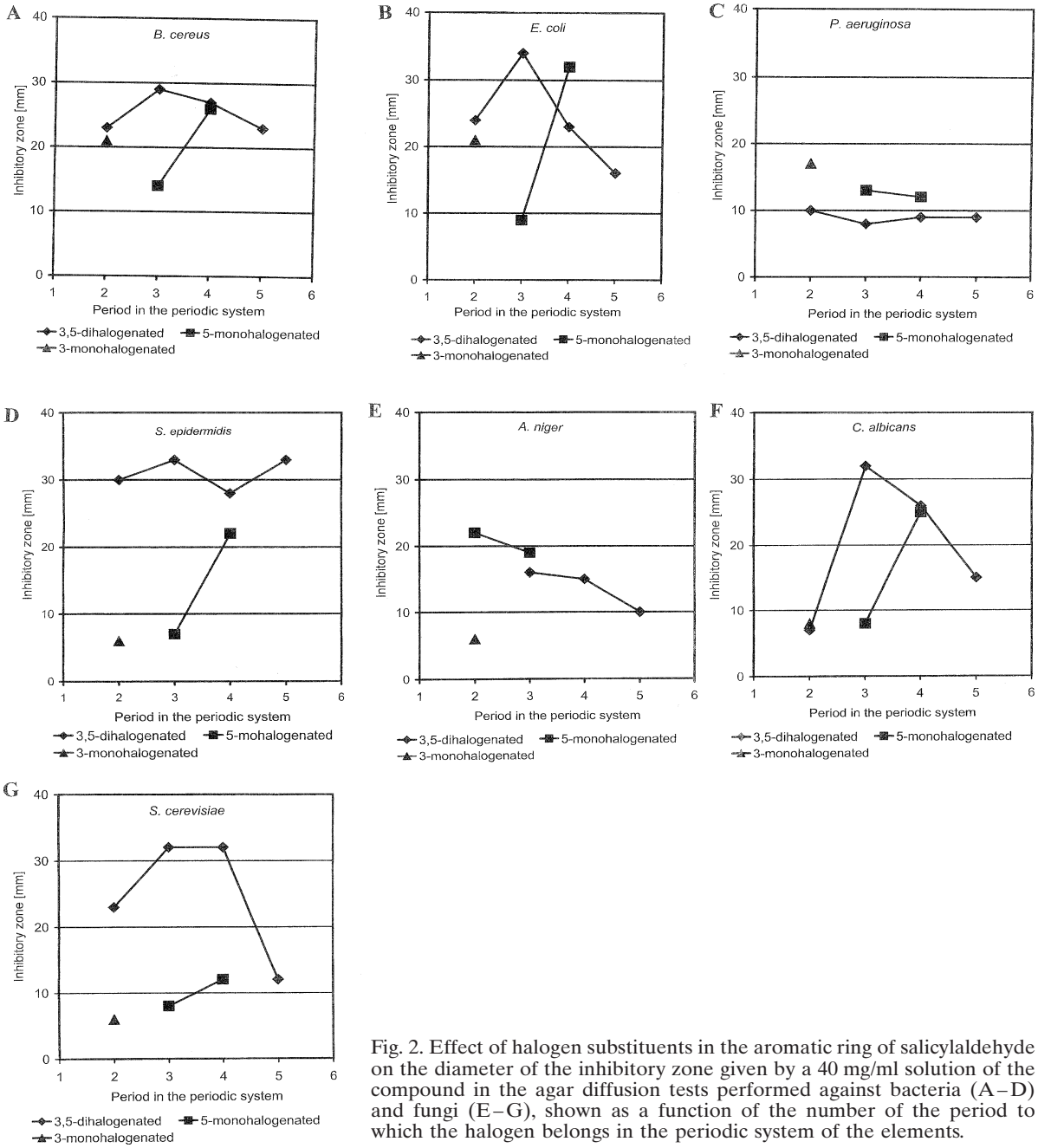


Fig. 2. Effect of halogen substituents in the aromatic ring of salicylaldehyde on the diameter of the inhibitory zone given by a 40 mg/ml solution of the compound in the agar diffusion tests performed against bacteria (A–D) and fungi (E–G), shown as a function of the number of the period to which the halogen belongs in the periodic system of the elements.

hydroxylated congeners had approximately similar activity that was, however, markedly lower than in the case of the other bacteria.

A very interesting result is constituted by the discovery that 2,3-dihydroxybenzaldehyde has very high activity against the yeasts *C. albicans* and *S. cerevisiae*, even the lowest concentration tested (5 mg/ml) producing inhibitory zones larger than 20 mm, while the other dihydroxylated congeners were completely inactive at this concentration.

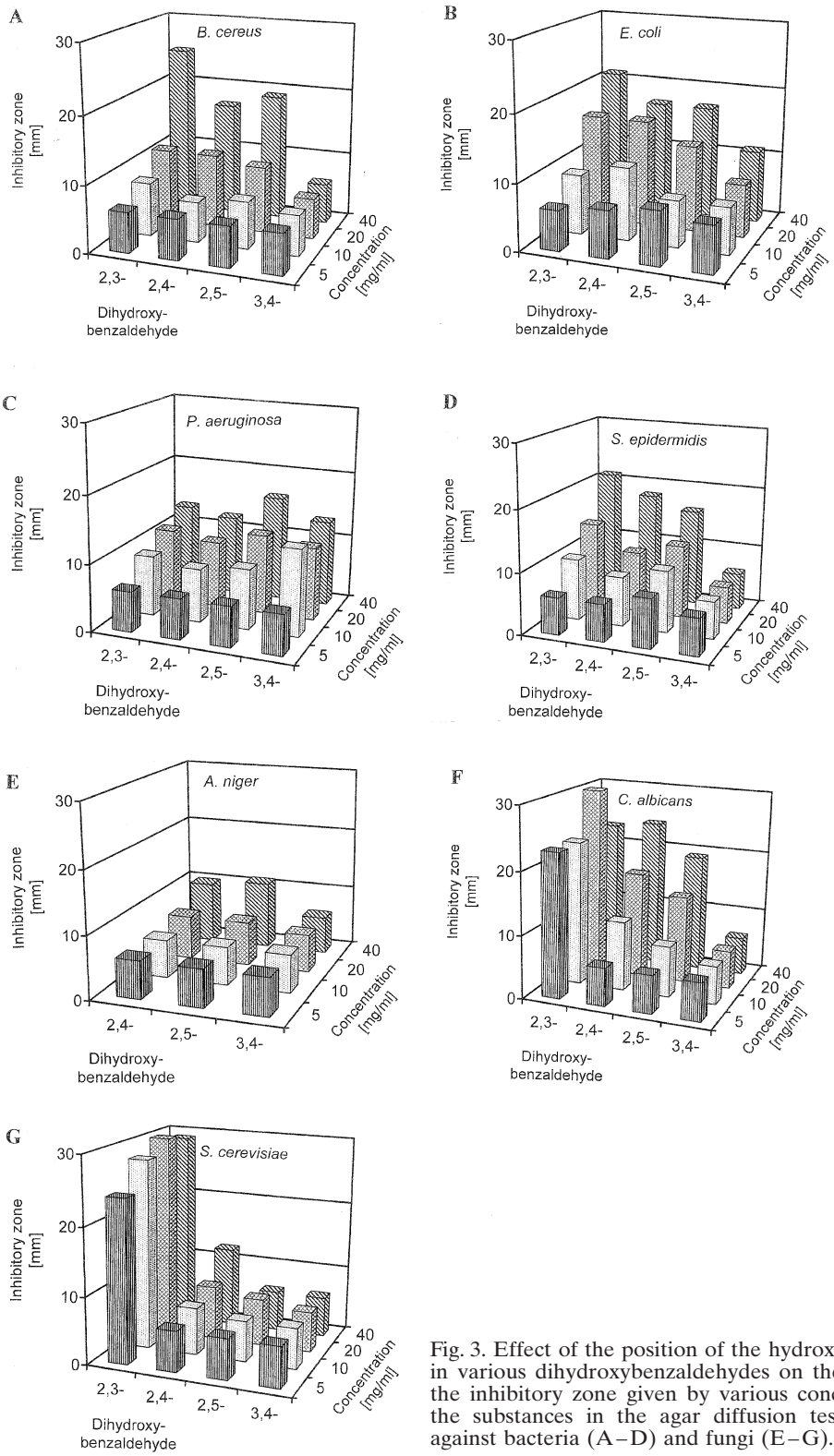


Fig. 3. Effect of the position of the hydroxy substituents in various dihydroxybenzaldehydes on the diameter of the inhibitory zone given by various concentrations of the substances in the agar diffusion tests performed against bacteria (A–D) and fungi (E–G).

Effects of nitro substituents on antimicrobial activity

5-Nitrosalicylaldehyde (**19**) was available from two commercial sources (Aldrich and TCI). Both samples displayed highly potent activity against *S. epidermidis*, even the lowest concentration tested producing inhibitory zone diameters as large as 34 mm (TCI) or 44 mm (Aldrich). Both lots showed potent activity also against the yeasts *C. albicans* and *S. cerevisiae* and noteworthy activity against all other microbes studied except for *P. aeruginosa*, against which both were practically inactive. The reasons for the differences between the lots remain speculative at present. One possibility is that the more active lot contained a highly active impurity (which does not seem probable) or that the less active lot contained a large amount of inactive impurities (which also seems improbable). More probably, however, the differences observed are at least in part due to experimental inaccuracy. In the case of very large inhibitory zones, even minimal changes in the experimental conditions may cause marked changes in the inhibitory zone diameters.

In contrast to 5-nitrosalicylaldehyde, 3,5-dinitrosalicylaldehyde (**20**) had minimal activity against most microbes studied, displaying marked activity only against *E. coli* and some activity against *B. cereus*. The reasons lying behind the remarkably lower activity of the dinitro compound, as compared to the 5-mononitro congener, remain obscure at present. We considered that one possibility might be that, in spite of being soluble in DMSO, the compound had been precipitated in the aqueous agar, apparently low activity thus being observed. This theory was considered to be supported by the fact that nitro substituents often drastically reduce the solubility of aromatic compounds. Therefore, we performed control experiments, in which solutions containing 40 mg of 5-nitrosalicylaldehyde (separate solutions for the TCI and Aldrich products) or 3,5-dinitrosalicylaldehyde per 1 ml of DMSO were added to either water or a liquid culture medium (YPD broth). When 25 μl of either one of the 5-nitrosalicylaldehyde solutions was added to 1 ml of water, a precipitate was immediately formed, while the same addition to YPD broth caused no precipitation. When the 3,5-dinitrosalicylaldehyde solution was similarly added to water or YPD broth, no precipitate was formed during a 30 min period of observation. The results were thus totally in contrast to expectations, and the lower activity of the dinitro compound, as compared to the mononitro congener, appears to be an intrinsic property of the compound.

Effects of other substituents on antimicrobial activity

One compound carrying a methyl group at the 6-position was also studied, namely 2,4-dihydroxy-6-methylbenzaldehyde (**9**). It was found to have a clearly lower antimicrobial activity than its non-methylated analogue 2,4-dihydroxybenzaldehyde (**4**), the last-mentioned compound displaying better inhibitory activity in the case of all microbial species studied and being a potent inhibitor of *C. albicans*, while the methylated congener had only modest activity. It can be speculated that the effect of the methyl substituent at the 6-position may possibly be due to steric hindrance in the vicinity of the aldehyde function. It would be interesting to investigate analogous compounds that carry the methyl substituent at another position or that have larger alkyl substituents or multiple substituents, but such compounds were not available for the present study.

One interesting compound studied is 2,4-dimethoxy-6-hydroxybenzaldehyde [*i. e.*, 4,6-dimethoxysalicylaldehyde (**10**)] that had considerable activity against *C. albicans* and slight activity against the other fungi studied, while displaying minimal activity against bacteria. As compared to salicylaldehyde that is devoid of the methoxy groups, the compound had dramatically higher antifungal activity. As compared to the close analogue 2,4,6-trihydroxybenzaldehyde (**8**) in which the 4- and 6-positions carry free hydroxy groups, the dimethoxy compound (in which those hydroxy groups are etherified) had a clearly different activity profile: the former compound had no activity against yeasts, having some activity against all bacteria studied, while the latter had selective anti-yeast activity. One further compound with an etherified hydroxy group, 5-(trifluoromethoxy)salicylaldehyde (**11**), was also studied but had low antimicrobial activity. Further studies on methoxylated and other alkoxylated aldehydes are in any case warranted.

One benzaldehyde derivative with an "exotic" substituent, 4-dimethylaminobenzaldehyde (**23**), was also studied but had minimal or no activity.

Antimicrobial activity of diformyl compounds (aromatic dialdehydes)

Among those aldehydes tested that do not contain a hydroxy group at the 2-position (*i.e.*, that are not salicylaldehydes), phthaldialdehyde [2-formylbenzaldehyde, 1,2-diformylbenzene (**22**)] displayed remarkable activity against *S. cerevisiae* even at the lowest concentrations tested and also had distinct activity against most other microbes studied, indicating that the 2-hydroxy substituent is not necessary for antimicrobial activity. However, it must be pointed out that the mechanism of action of phthaldialdehyde may be different from that of salicylaldehydes and may possibly be based on the high reactivity of the compound or on the presence of two reactive aldehyde functions that may give rise to the formation of cross-links between proteins or other biomolecules. In any case, the addition of the second formyl group to benzaldehyde has a prominent effect on the antimicrobial activity.

Also another dialdehyde (diformyl compound), 5-formylsalicylaldehyde (**21**), had remarkable antimicrobial activity. It displayed highest activity against *S. epidermidis* and was active against all other microbes as well, except *P. aeruginosa*. Thus, the 5-formyl substituent had a dramatic effect on the antimicrobial activity, since the unsubstituted parent compound salicylaldehyde is essentially inactive against all microbes tested.

In the light of the present results, further studies on the antimicrobial activity of aromatic dialdehydes are highly warranted.

Conclusions. Possible Practical Applications

The structure-activity relationships of the aldehydes studied are clearly different for different microbes, and the effects of substituents are not easily predicted nor can they be extrapolated from one microbe to another. The most active aldehydes had such high activity that they may be of practical value as such or as lead compounds for the development of drugs. Especially, activity against *C. albicans* and *A. niger* is noteworthy because of the need for new antifungal agents. For example in the case of the hereditary autoimmune endocrinopathy syndrome APECED (Buzi *et al.*, 2003), patients need long-standing anti-*Candida* therapy for mucocutaneous candidiasis, and the therapeutic options available are very limited. Another reason for the need of novel antifungal agents is constituted by the serious and even fatal side effects of current antifungal drugs (Elo, 2006; Shibata *et al.*, 2001; Magill *et al.*, 2004). Further studies on the aldehydes are thus highly warranted.

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