Carbohydrazones of Substituted Salicylaldehydes as Potential Lead Compounds for the Development of Narrow-Spectrum Antimicrobials

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Certain substituted salicylaldehydes are known to have highly potent antimicrobial activity against bacteria and fungi, but the mechanism underlying this remarkable activity is not known, and almost nothing has been reported on the effects of further modification of the structures, such as the formation of hydrazone-type derivatives. We report now a study on the antimicrobial properties of the carbohydrazone derivatives of several substituted salicylaldehydes. The compounds studied were synthesized from ring-substituted salicylaldehydes and carbohydrazide in the mole ratio 2:1. They were tested against *Aspergillus niger*, *Bacillus cereus*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* and *Staphylococcus epidermidis* using the agar diffusion method. The carbohydrazone derived from 2,3,4-trihydroxybenzaldehyde had distinctly higher activity than the parent aldehyde in the same molar concentration. This activity was limited to one test organism (*S. epidermidis*), while the free aldehyde had at least some (in some cases even high) activity against all of the microbes studied. All other ones of the effective carbohydrazone compounds were distinctly less active than the parent salicylaldehydes as such. The hydrazones studied had in general a narrower antimicrobial spectrum than the free aldehydes and are thus of interest as potential lead compounds for the development of narrow-spectrum antimicrobial drugs. The mechanism of action of the aldehydes as well as that of the carbohydrazones is discussed.

Key words: Antibacterial Antifungal Agents, Hydrazones, 2-Hydroxybenzaldehyde Analogues, Substituent Effects

Introduction

Certain substituted salicylaldehydes are known to have highly potent antimicrobial activity against certain bacteria and fungi (Bougault *et al.*, 1949; Burton *et al.*, 1964a, b, 1965; Clarke *et al.*, 1963; Cronenberger *et al.*, 1968a, b, 1969; Rehn *et al.*, 1981; Taillandier and Pera, 1991; Pelttari *et al.*, 2007). 3,5-Dihalogenated and nitro-substituted congeners are especially effective, while salicylaldehyde itself is practically inactive. The ultimate mechanism underlying the remarkable antimicrobial activity is not known, and almost nothing appears to have been reported on the effects of further modification of the structures, such as the formation of hydrazone-type derivatives of the highly reactive aldehydes. On the other hand, certain hydrazone derivatives of salicylaldehyde itself are known to have antimicrobial activity. These substances include, for example, the well-known tuberculostatic agent salinazid (1-isonicotinoyl-2-salicylidenehydrazone, also known as Nupa-Sal or Acozid) (Bavin *et al.*, 1955) as well as some antitubercular aryl-substituted thiosemicarbazones of salicylaldehyde (Wahab, 1978). Therefore, we have performed a study on the antimicrobial properties of the carbohydrazone derivatives of several substituted salicylaldehydes (see Fig. 1 for the structures), the results of which are reported in this article.

Experimental

The carbohydrazone compounds were synthesized from the corresponding salicylaldehydes and carbohydrazide (mole ratio 2:1). Details of the syntheses will be described elsewhere. Because of solubility problems, the compounds were difficult

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to purify and may have contained small amounts of starting materials or by-products, in which only one of the two hydrazine moieties of carbohydrazide reacted with the aldehyde.

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 as well as the experimental procedures employed have been described previously (Pelttari *et al*., 2002). In brief, for testing of the antimicrobial activities, single colonies of the microbes from agar plates were 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 were 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

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*) were employed as test organisms. The results obtained are shown in Table I. As is evident from Table I, the carbohydrazone compound derived from 2 mol of 2,3-dihydroxybenzaldehyde and 1 mol of carbohydrazide (**Ia**; Fig. 1) had distinct and dose-dependent antimicrobial activity against *C. albicans* and slight activity against *E. coli*, but was inactive against the other microbial strains. On the other hand, this compound was the only one that inhibited the growth of these two microbial strains. The apparently similar behaviour of these two species is a curious result, as one of the two species is a yeast and the other one a prokaryote.

The carbohydrazone compound derived from 2 mol of 2,3,4-trihydroxybenzaldehyde and 1 mol of carbohydrazide (**Ib**) had distinct and dose-depend-

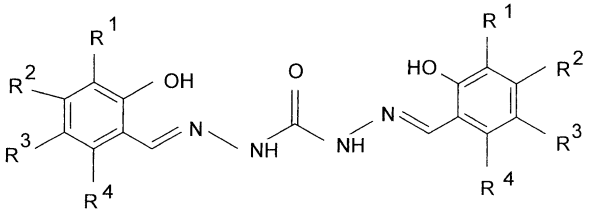
Table I. Results of growth inhibition tests.[a]

Compound	Concentration [μM][c]	Concentration [mg/ml][c]	Mean diameter of inhibitory zone [mm][b]			
			B. cereus	*E. coli*	*S. epidermidis*	*C. albicans*
Ia	121	40		8		12
	60	20		7		11
	30	10		8		10
	15	5	8	8		10
Ib	110	40			12	
	55	20			9	
	27	10			7	
	14	5				
Ie	115	40	9		9	
	57	20	10		9	
	29	10	9		9	
	14	5	9		9	
Ig	65	40	9		9	
	32	20	9		8	
	16	10	9		8	
	8	5	9		8	

[a] Results obtained for compounds **Ic**, **Id**, **If** and **Ih** are not shown, since these compounds were inactive in all cases. Results obtained using *P. aeruginosa*, *S. cerevisiae* and *A. niger* are also omitted, since none of the compounds tested had activity against these microbes.

[b] 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. In most cases, the same result was obtained for all four discs, and if not, the range of the results was usually 1 mm (maximum 3 mm). Blank space (instead of a diameter) indicates inactive compound.

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



Ia: R^1 = OH, R^2 = H, R^3 = H, R^4 = H
Ib: R^1 = OH, R^2 = OH, R^3 = H, R^4 = H
Ic: R^1 = H, R^2 = OH, R^3 = H, R^4 = OH
Id: R^1 = H, R^2 = H, R^3 = Cl, R^4 = H
Ie: R^1 = Cl, R^2 = H, R^3 = Cl, R^4 = H
If: R^1 = H, R^2 = H, R^3 = Br, R^4 = H
Ig: R^1 = Br, R^2 = H, R^3 = Br, R^4 = H
Ih: R^1 = H, R^2 = H, R^3 = NO_2, R^4 = H

Fig. 1. The structures of the salicylaldehyde derivatives studied.

ent antimicrobial activity against *S. epidermidis*, but not against any other of the microbes studied. This result is interesting, since the compound is closely related to the one derived from 2,3-dihydroxybenzaldehyde and carbohydrazide.

We also attempted to prepare another closely related substance, the carbohydrazone compound derived from 2 mol of 2,4,6-trihydroxybenzaldehyde and 1 mol of carbohydrazide (**Ic**), but the product obtained provided neither acceptable NMR spectra nor acceptable analytical results and did not have activity against any of the microbes studied. Previously, similar synthetic difficulties have been encountered with the synthesis of the copper(II) chelate of the oxime of this aldehyde, while the corresponding syntheses of derivatives of other di- and trihydroxybenzaldehydes have been straightforward. These differences may possibly be due to oxidation of the 2,4,6-trihydroxy compound(s).

The carbohydrazone compound derived from 2 mol of 2-hydroxy-5-chlorobenzaldehyde and 1 mol of carbohydrazide (**Id**) was completely inactive against all of the microbes studied, while the monohydroxy dichloro analogue derived from 2 mol of 2-hydroxy-3,5-dichlorobenzaldehyde and 1 mol of carbohydrazide (**Ie**) had slight activity against *B. cereus* and *S. epidermidis* (but not against the other microbes investigated). The activity was distinct even when the concentration of the solution applied on the disc was only 5 mg/ml, but it did not increase on increasing concentration. The bromo analogue of the monochloro compound (**If**) was also synthesized and was completely inactive against all of the microbes studied, just like the monochloro congener. The dibromo analogue of the dichloro compound (**Ig**) was likewise synthesized and, just like the dichloro compound, was found to have activity against *B. cereus* and *S. epidermidis* (but not against the other microbes investigated). This activity was slightly lower than that of the dichloro congener. Unfortunately, the difluoro and diiodo analogues were not available for testing.

One nitrosalicylaldehyde derivative (**Ih**) was also prepared. Just like the corresponding monochloro and monobromo congeners, this compound was completely inactive against all of the microbes studied. Unfortunately, dinitro analogues were not available for this study.

Although some of the carbohydrazone compounds derived from 2 mol of salicylaldehydes (substituted 2-hydroxybenzaldehydes) and 1 mol of carbohydrazide had distinct antimicrobial activity, only one of them (**Ib**) had higher activity than the parent aldehyde in the same concentration (mg/ml). This activity was limited to one test organism (*S. epidermidis*), while the free aldehyde had at least some activity against all of the microbes studied, and it was actually highly active against some of them. All other of the effective carbohydrazone compounds were distinctly less active than the parent salicylaldehydes as such (data on aldehydes not shown; see Pelttari *et al.*, 2007). Those salicylaldehydes that are structural fragments as well as starting materials of the antimicrobially active carbohydrazones now studied had in all cases a wider antimicrobial spectrum than the carbohydrazones. A comparison of activities on a molar concentration basis indicates a distinctly higher activity of compound **Ib** as compared to the free aldehyde. The free aldehydes and a large number of their analogues have been tested in our laboratory against the same microbes as were used in the present study. A detailed report on those results is given separately (Pelttari *et al.*, 2007).

The other structural portion and starting material of the carbohydrazones now studied, carbohydrazide (in concentrations of 5, 10, 20 and 40 mg/ml), was completely inactive against *C. albicans*, *S. cerevisiae* and *S. epidermidis*, while it showed activity against *B. cereus* and *E. coli* at concentrations of 20 and 40 mg/ml, and against *P. aeruginosa* at all of the concentrations tested. This difference in its antimicrobial spectrum, as compared to the

carbohydrazones tested, and especially its fairly high activity against *P. aeruginosa*, against which *all* of the carbohydrazones were inactive, obviously does not suggest a common mechanism of action.

Whether the carbohydrazones exert their activity by the same mechanism(s) as the free aldehydes or not, remains to be investigated. One possibility is that the carbohydrazones act by releasing the aldehydes or by exchange of the carbohydrazide moiety and an amino moiety (*i.e.*, by formation of Schiff bases with amino groups of vital components of the microbes such as proteins). Interestingly, however, salicylaldehyde itself is practically devoid of antimicrobial activity, although it also forms Schiff bases. Also release of carbohydrazide and a subsequent reaction of it with important carbonyl groups is one possibility.

A prominent feature of the present results is constituted by the discovery that the carbohydrazones of 3,5-dihalogenated (*i.e.*, 3,5-dichloro and 3,5-dibromo) salicylaldehydes are only effective against such microbes that are resistant towards the carbohydrazone of 2,3-dihydroxybenzaldehyde, and *vice versa*.

In conclusion, although most compounds synthesized had low or no activity, the present and analogous carbohydrazone compounds are worth further studies, as the effective ones seem to have a much narrower antimicrobial spectrum than the free aldehydes have. This may be of great interest concerning the development of narrow-spectrum antimicrobials. Furthermore, the higher activity of carbohydrazone **Ib** against *S. epidermidis*, as compared to its aldehyde component, suggests that further studies are worthwhile. Also testing against larger panels of microbes is warranted. In addition, analogous compounds comprising another hydrazine derivative instead of carbohydrazide are worth synthesis and antimicrobial testing.

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