PAPULACANDINS — THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND EFFECT ON GLUCAN SYNTHESIS IN YEAST

GÜNTER RÖMMELE, PETER TRAXLER and WALTER WEHRLI

Pharmaceutical Research Laboratories, Ciba-Geigy Limited,
CH4002, Basel, Switzerland

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Papulacandin B inhibits glucan biosynthesis in cells of *Saccharomyces cerevisiae* and *Candida albicans*. Biological studies with a series of papulacandin derivatives showed that the short fatty acid chain and the galactose residue are not required for activity at the target site, but that they can affect penetration. On the other hand, the long fatty acid residue is essential for biological activity.

The papulacandins are a family of antibiotics which were isolated from a strain of *Papularia sphaerosperma* (Pers.), Hoehnel and which strongly inhibit the growth of *Candida albicans*[1]. The chemical structures of the papulacandins A, B, C and D have been elucidated[2,3]. In addition, a large number of chemical derivatives have been synthesized (P. TRAXLER, in preparation).

Papulacandin B strongly affects glucan biosynthesis in yeast spheroplasts[4]. DURÁN and coworkers showed that, in the fungus *Geotrichum lactis*, papulacandin B inhibits the enzyme 1,3-β-D-glucansynthase[5]. The corresponding enzyme from *S. cerevisiae* was only slightly inhibited by high concentrations of the drug (E. CABIB, personal communication). Thus the precise mechanism of action of the papulacandins remains to be elucidated.

The natural papulacandins, their degradation products and semisynthetic derivatives differ quite strongly in their ability to inhibit the growth of *Candida albicans*. Such differences could be due to varying degrees of activity at the site of action. Alternatively, other factors, such as degradation of compounds during microbial testing, or differences in the rate of penetration into the fungal cell could play an important role.

In this paper, the structural requirements of the papulacandin molecule for activity at the target site have been investigated.

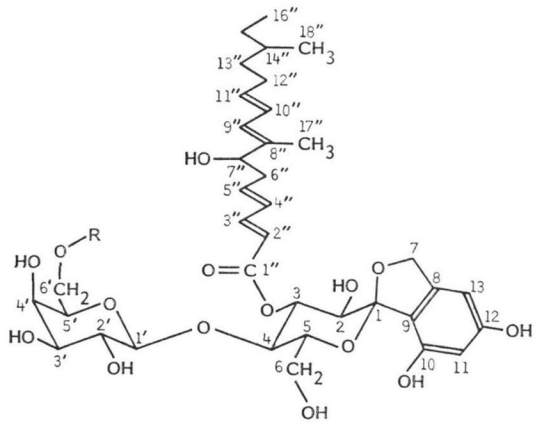
Materials and Methods

The papulacandin derivatives described here have either been isolated from cultures of *Papularia sphaerosperma* or synthesized in our laboratories from papulacandin B. To measure glucan biosynthesis, *Candida albicans* K 1133, a standard test strain of our laboratories was used. The growth of the cells, the preparation and incubation of spheroplasts, the measurement of the glucan synthesis and the growth inhibition of *Candida albicans* K 1133 by the various derivatives were carried out as described in detail by BAGULEY *et al.*[4].

Results

As shown in Fig. 1 the structures of papulacandins A, B and C consist of a diglucoside nucleus containing a galactose moiety connected by a β-1,4-linkage to a glucose residue. This diglucoside is further connected with an aromatic ring *via* C-O and C-C bonds forming a spirocyclic system.

Fig. 1. Structures of papulacandins A, B and C.



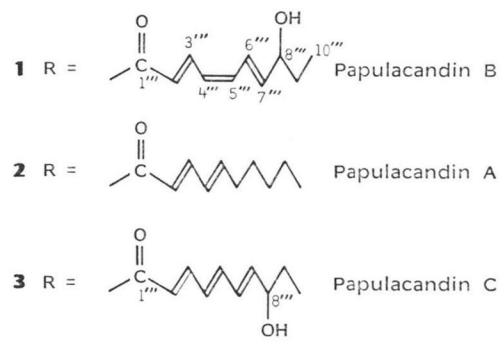


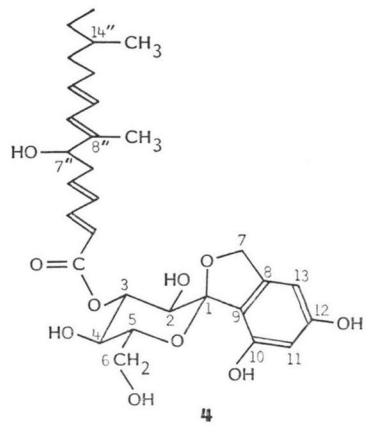
Table 1. Papulacandin derivatives with variations in the fatty acids and/or the galactose residue.

	Compound	ED_{50} glucan synthesis* (μg/ml)	MIC** (μg/ml)
1	Papulacandin A	0.05	0.2
2	Papulacandin B	0.05	0.1
3	Papulacandin C	0.15	0.4
4	Papulacandin D	0.13	1~2
5	Tetradecahydropapulacandin B	0.6	>128
6	Octahydropapulacandin D	3	>128
7	Papulacandin B nonaacetate	>5	>128
8	Degradation product I	0.3	>128
9	Degradation product II	>5	>128
10	Degradation product III	>5	>128

* 50% inhibition of glucan synthesis in spheroplasts.

** Minimum inhibitory concentrations for *Candida albicans*.

Fig. 2. Structure of papulacandin D.



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Two unsaturated fatty acids are attached to the diglucoside nucleus by ester-bonds, a C_{16}-acid to position 3 of the glucose residue and a C_{10}-acid to position 6′ of the galactose moiety.

Papulacandin Derivatives with Variations in the Fatty Acids and/or the Galactose Residue

From Table 1 it is evident that papulacandin B[2] is the most active compound both in growth inhibition (MIC) and inhibition of glucan biosynthesis. Slight structural changes of the short fatty acid (papulacandin A (**1**) or papulacandin C (**3**)) have only a minor effect on the biological activity. Hydrogenation of both fatty acid residues (compound **5**) or removal of the short fatty acid (compound **8**, see Fig. 3) does not greatly affect the action on glucan biosynthesis, whereas growth inhibition is no longer found (MIC >128 μg/ml). Papulacandin D (**4**, Fig. 2), a derivative without short fatty acid and galactose residue, is still a quite active compound at both the target site and in respect to its MIC. On the other hand hydrogenation of papulacandin D (compound **6**) clearly reduces the biological activity. Removal of both fatty acid residues (compound **9**) or the additional removal of the galactose moiety (compound **10**, see Fig. 3) leads to a total loss of activity. The same is true for papulacandin B nona-

Fig. 3. Degradation products of papulacandin B.

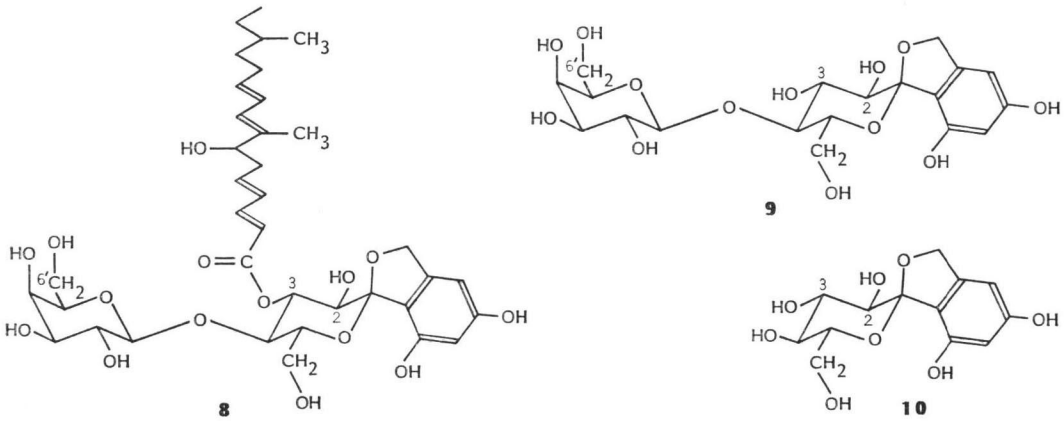

Table 2. Derivatives of papulacandin B with modifications in the spirocyclic diglucoside.

	Structure	ED_{50} glucan synthesis (μg/ml)	MIC (μg/ml)
	Derivatives in position 6 of glucose		
11	6-I	0.5	0.2
12	6-Br	0.5	0.2
13	6-OCH_2COCH_3	0.5	0.5
14	6-CH_2N O	0.2	2
	Derivatives in position 10, 11 and 12 of the aromatic ring		
15	12-OCH_3	0.3	0.4
16	10,12-OCH_3	0.5	0.8
17	12-$OCH_2CH_2CH_3$	2	12
18	10,12-$OCH_2CH_2CH_3$	>5	>128
19	10-$OCH_2C(=O)CH_3$	0.1	0.2
20	12-OCH_2COOH	3	6
21	10-OCH_2COOH	0.5	0.2
22	10-OCH_2COOCH_3	0.3	0.2
23	10-$OCH_2COOCH_2CH(OH)CH_2OH$	0.3	0.5
24	11-CH_2N O, 12-OCH_3	1	6
25	11-CH_2N NH	3	1.6
26	11-NH_2, 12-OCH_3	0.2	0.5
27	11-$NHCOCH_3$, 12-OCH_3	0.5	2
28	11-$NHCOCH_3$	0.5	0.2
29	11-$NHCOCH_2NH_2$	0.5	1

acetate (compound **7**).

Derivatives of Papulacandin B with Modifications in the Diglucoside Nucleus

None of the derivatives tested shows a better activity than papulacandin B in terms of MIC or in-

hibition of glucan synthesis. Variations in position 6 of the glucose (compounds **11**~**14**) or in position 10 of the aromatic ring (10-methyl ethers, compounds **16**, **19**, **21**~**23**) have no great effect on the biological activity. When substituents in position 12 are larger than a methyl group (compounds **17**, **18** and **20**) a clear loss of effect on glucan synthesis and fungal growth results. Substitutions in position 11 lead to a more or less pronounced loss of biological activity (compounds **24**~**29**).

Conclusions

The biological analysis of a series of papulacandin derivatives shows that clear differences exist between the structural requirements for effective action at the target site of the drugs, namely the inhibition of the glucan biosynthesis, and the reaching of the target site, a parameter important for the inhibition of fungal growth (MIC). The following conclusions can be made from the results:

The presence of the long fatty acid residue is an absolute requirement for activity at the target site. Removal of this residue from the degradation product **8**, which is still active at the target site, gives the inactive degradation product **9**.

The results obtained with papulacandin D (**4**) prove that neither the presence of the short fatty acid nor the galactose moiety are essential for biological activity. Apparently the degradation product **8** does not reach the target site.

Hydrogenation of the fatty acids (compounds **5** and **6**) leads to a loss of activity. In this case, penetration to the target site seems to be more strongly influenced.

The derivatives synthesized so far with substitutions in the diglucoside nucleus show activities in most cases similar to those of papulacandin B.

These data lead one to conclude that less complex derivatives such as papulacandin D may be good starting material for further chemical modifications to obtain more active compounds.

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