PAPULACANDINS — SYNTHESIS AND BIOLOGICAL ACTIVITY OF PAPULACANDIN B DERIVATIVES

PETER TRAXLER, WERNER TOSCH and OTO ZAK

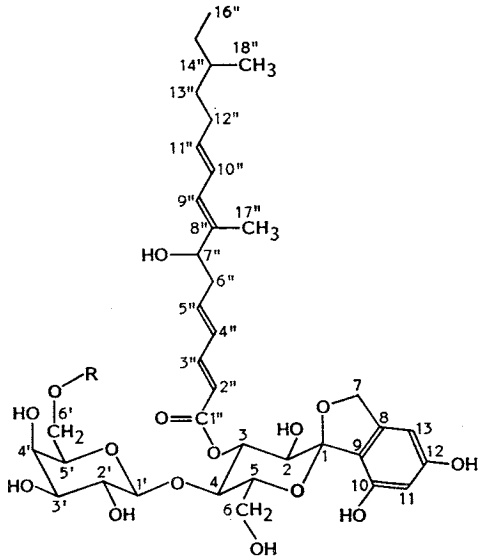
Research Department, Pharmaceuticals Division, CIBA-GEIGY Limited, Basel, Switzerland

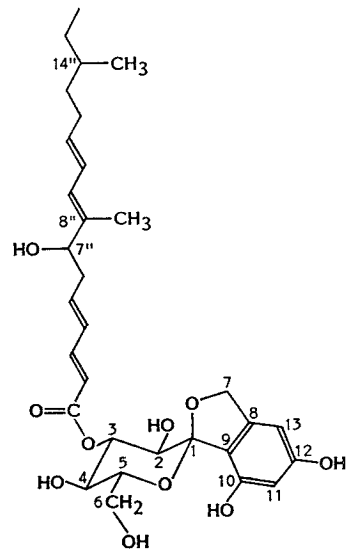
(Received for publication March 30, 1987)

A series of papulacandin B derivatives was synthesized and their *in vitro* and *in vivo* activity against *Candida albicans* and other fungi was established. The biological data have shown that some 10-alkyl ether and 11-acylamino derivatives exhibit an improved *in vivo* activity compared to papulacandin B whereas derivatization in other positions of the molecule led to less potent compounds.

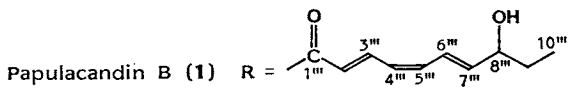
Some years ago, a group of antibiotics with a novel structure, the papulacandins, was isolated from cultures of *Papularia sphaerosperma*[1,2]. The structures of the principal component papulacandin B and the accessory components A, C and D (Fig. 1) were subsequently elucidated by degradation reac-

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

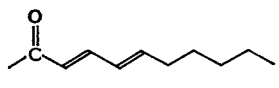




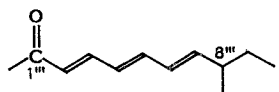
Papulacandin D (4)



Papulacandin B (1) R =



Papulacandin A (2) R =



Papulacandin C (3) R =

tions and spectroscopic analysis of the natural and the degradation products[3,4]. The papulacandins all contain a spirocyclic diglycoside[5] and two long-chain unsaturated fatty acids, linked as esters with two hydroxyl groups of the diglycoside (Fig. 1). Recently, chaetiacandin was isolated from the mycelia of *Monochaetia dimorphospora*. The structure of this substance is very closely related to that of the papulacandins, but in chaetiacandin the spirocycle is opened[6,7].

The papulacandins display a high degree of activity against *Candida albicans* and various other yeasts. They are, however, only weakly active, or inactive, against other fungi, bacteria and protozoa. Papulacandins have been shown to inhibit glucan synthesis in yeast spheroplasts[8], in *Geotrichum lactis*[9,10] and in *Schizosaccharomyces pombe*[11].

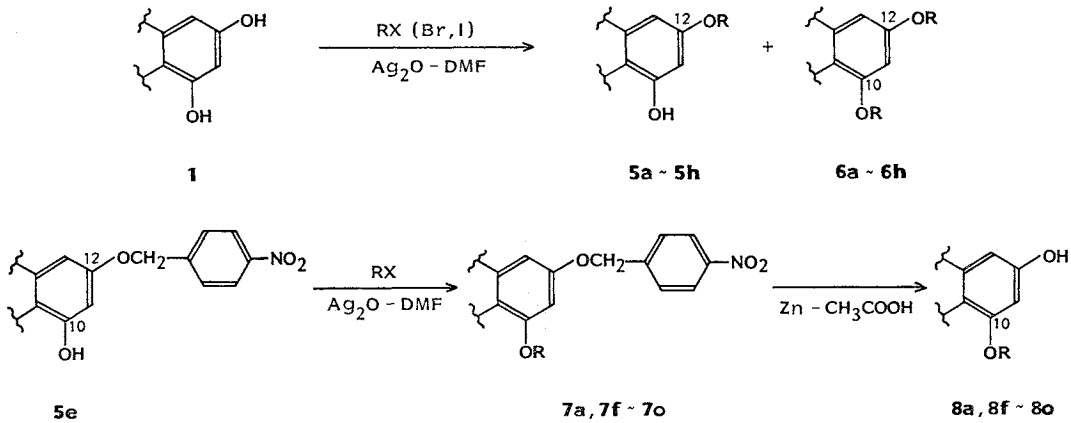
Although highly active *in vitro*, the papulacandins are much less effective *in vivo*. With a view to improve their *in vivo* activity, and to gain information on their structure-activity relations, a relatively large series of derivatives has been synthesized.

Chemistry

Degradation Reactions and Hydrogenation Products

The basic hydrolysis of papulacandin B (**1**) to a spirocyclic diglycoside and two fatty acids and the selective basic hydrolysis as well as hydrogenation of **1** have already been described in connection with

Scheme 1. Ether derivatives of papulacandin B (**1**).



Compound	R
a	CH_3
b	CH_2CH_3
c	$CH_2CH_2CH_3$
d	$CH_2CH_2CH_2I$
e	CH_2–C$_6$H$_4$–NO_2
f	CH_2COCH_3
g	CH_2COOH
h	CH_2COOCH_3
i	$CH_2COOCH_2CH_3$
k	$CH_2COOCH_2CH(OH)CH_2OH$
l	CH_2CONH_2
m	$CH_2CONHCH_3$
n	$CH_2CON(CH_3)_2$
o	CH_2CO–C$_4$H$_3$S

the elucidation of its structure.

Ether Derivatives

The two phenolic hydroxyl groups in papulacandin B (**1**) are readily etherified with alkylhalogenides in the presence of silver oxide. Depending on the reaction conditions, a mixture of 12-monoethers and 10,12-diethers is formed (Scheme 1, compounds **5a**~**5h** and **6a**~**6h**).

Starting from 12-papulacandin B-*p*-nitrobenzyl ether (**5e**), a series of 10-alkyl ether derivatives was prepared by further alkylation in position 10 and subsequent cleavage of the protecting group (Scheme 1, compounds **8a**, **8f**~**8o**).

To prepare the acid derivative **8g**, **5e** was alkylated with bromacetic acid-*p*-nitrobenzyl ester and converted by cleavage of the two protecting groups to the desired acid **8g**.

Aminomethylene Derivatives

Positions 11 and 13 of the aromatic ring are activated for nucleophilic substitutions. By way of Mannich reactions, basic groups can be introduced which are suitable for salt formation and thereby could improve the solubility of the antibiotic in water.

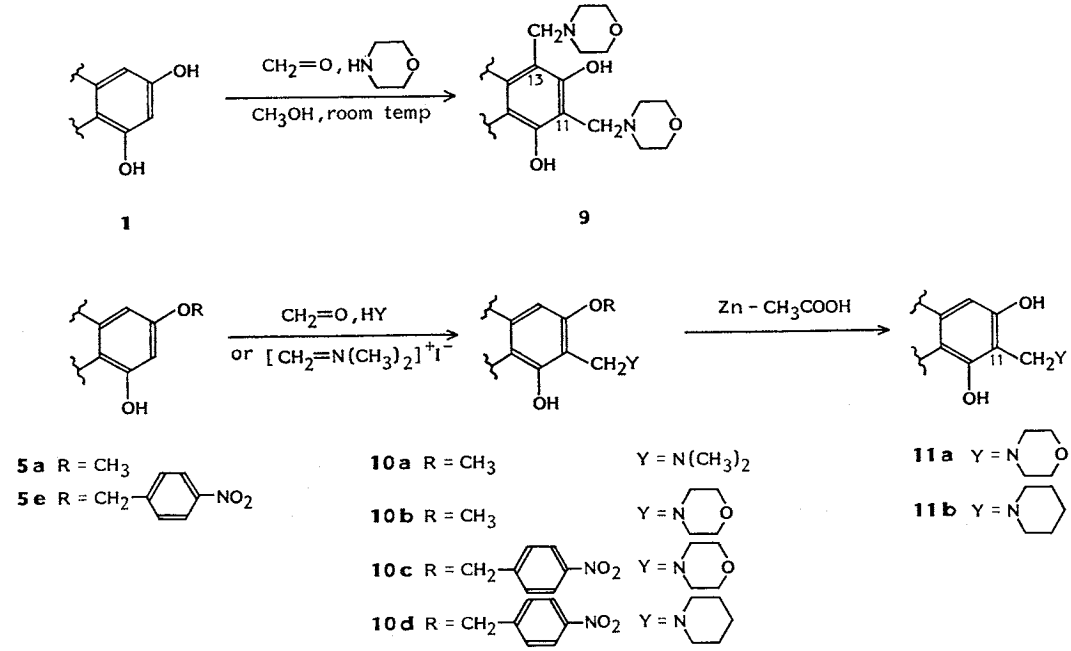
Upon reaction of **1** with an excess of formaldehyde and morpholine, the 11,13-dimorpholinomethylene compound **9** is formed (Scheme 2).

If, instead of **1**, its 12-methyl ether **5a**, or the 12-nitrobenzyl ether **5e**, is used, with formaldehyde and secondary bases or the use of dimethylaminomethylene iodide to introduce the dimethylaminomethylene residue the 11-aminomethylene derivatives **10a**~**10d** are formed. Cleavage of the *p*-nitrobenzyl protecting group from **10c** and **10d** yields the 11-aminomethylenepapulacandins **11a** and **11b** with free phenolic hydroxyl groups (Scheme 2).

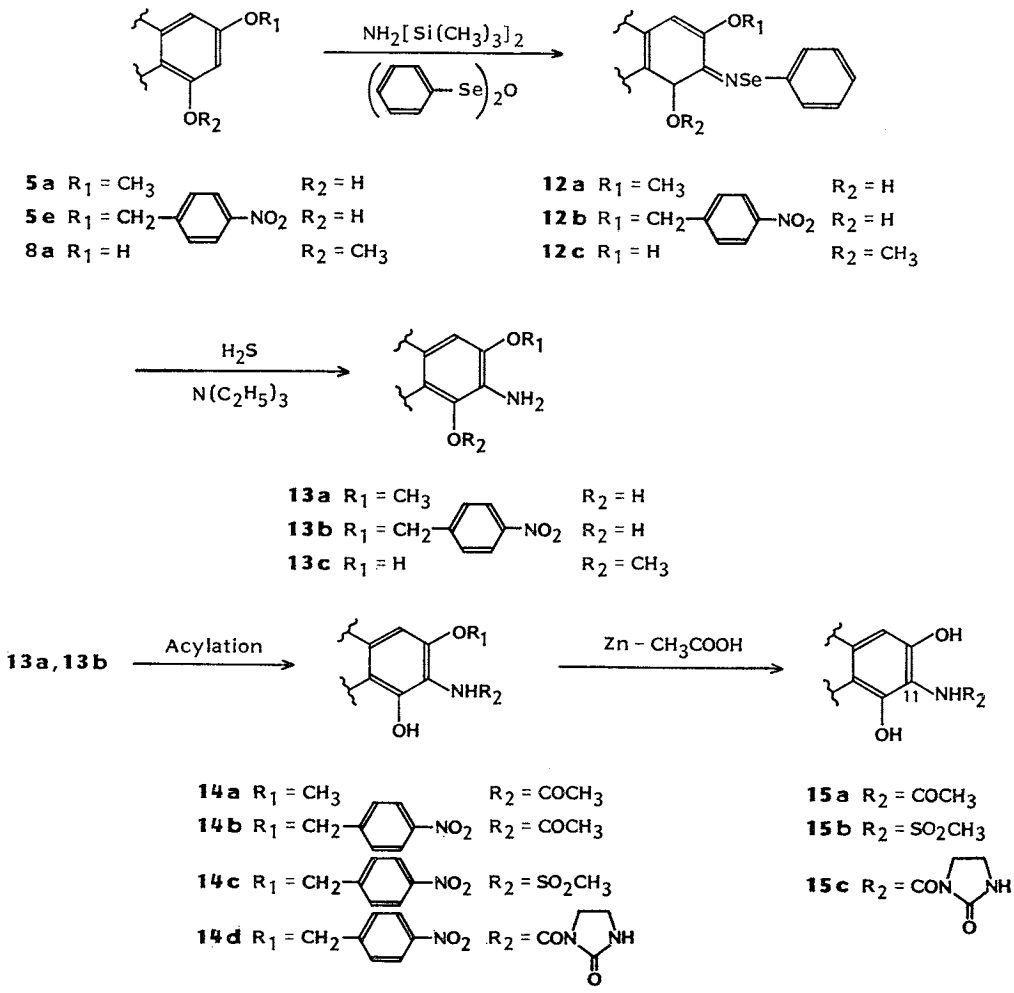
Aromatic Amines in Position 11

According to a method described by BARTON *et al.* amino groups can be smoothly introduced into

Scheme 2. Aminomethylene derivatives of papulacandin B (1).



Scheme 3. 11-Aminopapulacandin B derivatives.



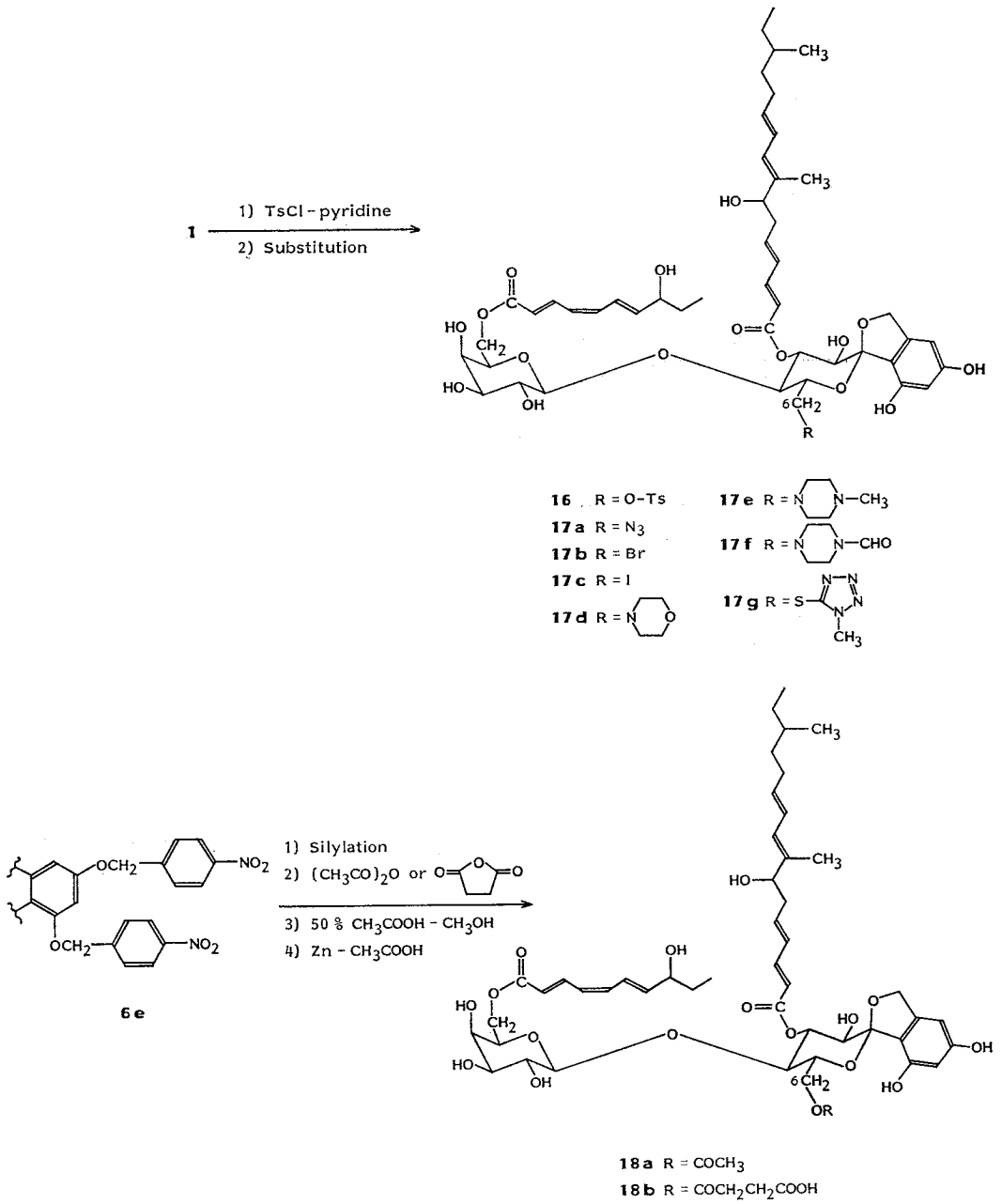
phenoles by using diphenylseleno anhydride and hexamethyldisilazane *via* phenylseleno imines[12]. This method proved unsuccessful with papulacandin B (**1**); but with the papulacandin B-12-methyl ether (**5a**) or its 12-nitrobenzyl ether (**5e**), the desired aromatic amines **13a** and **13b** were obtained. Removal of the protecting group from **13b** with zinc acetic acid, however, yielded no defined products (Scheme 3). Similarly, the 11-aminopapulacandin B-10-methyl ether (**13c**) was produced from papulacandin-10-methyl ether (**8a**) (Scheme 3).

The two aromatic amines **13a** and **13b** can be used as starting substances for acylations. Acetylation of **13a** yielded the aminoacetate **14a**. Various acylations were performed with the 11-aminopapulacandin B-12-*p*-nitrobenzyl ether (**13b**). After removal of the respective protecting group the 11-aminoacyl compounds **15a**~**15c** with free phenolic hydroxyl groups were obtained (Scheme 3).

Derivatives in Position 6 of the Glucose Moiety

The primary hydroxyl group in position 6 of the diglycoside moiety of **1** can be selectively converted to the tosylate **16**. **16** is well suited for use as a starting substance for further substitution reactions (compounds **17a**~**17g**, Scheme 4).

Scheme 4. Papulacandin B derivatives in position 6 of glucose.

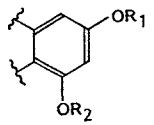


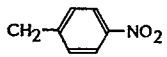
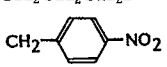
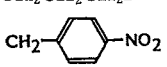
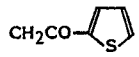
If papulacandin B-10,12-dimethyl ether (**6e**) is persilylated according to a method of McINNES[13] and FUCHS and LEHMANN[14], and acetylated in pyridine - acetic acid anhydride or succinic acid anhydride with addition of catalytic amounts of acetic acid, after removal of the protecting group 6-acetylpapulacandin B (**18a**), or 6-succinylpapulacandin B (**18b**) is obtained (Scheme 4).

Biology

All degradation products of **1** and their hydrogenation products are biologically inactive (data not

Table 1. Antifungal activity *in vitro* (MIC) and *in vivo* (ED_{50}) of ether derivatives of **1**.

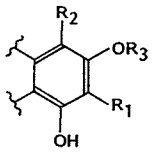
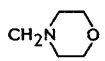
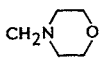
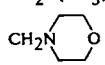
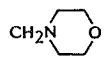
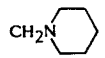


Compound	R_1	R_2	MIC (μg/ml) *C.a.* K 1133	*C.a.* K 1082	*C.a.* K 75	*C.t.* ATCC 13803	*A.f.* ATCC 9197	*S.s.* ATCC 10212	*T.m.* ATCC 9533	*M.c.* ATCC 10214	ED_{50} (mg/kg, mice) *C.a.* K 1133 4×sc
1	H	H	0.05	0.1	0.1	0.2	100	100	100	0.1	80
5a	CH_3	H	0.4	0.4	0.4	0.8	>100	>100	12.5	1.6	120
5b	CH_2CH_3	H	3.1	3.1	3.1	6.2	>100	>100	6.2	1.6	>100
5c	$CH_2CH_2CH_3$	H	12.5	12.5	12.5	50	>100	>100	12.5	6.2	>300
5d	$CH_2CH_2CH_2I$	H	6.2	6.2	6.2	12.5	>100	>100	6.2	3.1	>300
5e		H	3.1	3.1	3.1	6.2	>100	>100	3.1	1.6	>300
5f	CH_2COCH_3	H	3.1	3.1	3.1	6.2	100	100	25	0.8	140
5g	CH_2COOH	H	6.2	50	>100	>100	>100	>100	>100	12.5	>300
5h	CH_2COOCH_3	H	1.6	1.6	1.6	1.6	50	50	25	0.8	180
6a	CH_3	CH_3	0.8	0.8	0.8	1.6	100	100	12.5	0.8	>300
6c	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	>100	>100	>100	>100	>100	>100	>100	>100	nt
6d	$CH_2CH_2CH_2I$	$CH_2CH_2CH_2I$	>100	>100	>100	>100	>100	>100	>100	>100	nt
6e			>100	>100	>100	>100	>100	>100	>100	>100	nt
6f	CH_2COCH_3	CH_2COCH_3	6.2	6.2	6.2	6.2	>100	>100	12.5	0.8	100
6g	CH_2COOH	CH_2COOH	25	100	100	100	>100	>100	>100	50	>300
6h	CH_2COOCH_3	CH_2COOCH_3	12.5	12.5	12.5	12.5	>100	>100	100	6.2	>300
8a	H	CH_3	0.2	0.1	0.1	0.2	>100	>100	>100	0.1	300
8f	H	CH_2COCH_3	0.1	0.2	0.2	16	>100	>100	12.8	12.8	30
8g	H	CH_2COOH	0.2	0.5	0.5	2	12.8	>100	12.8	12.8	60
8h	H	CH_2COOCH_3	0.1	0.2	0.2	1	>100	>100	12.8	12.8	50
8i	H	$CH_2COOCH_2CH_3$	0.5	1	0.5	1	nt	nt	nt	nt	>300
8k	H	$CH_2COOCH_2CH(OH)CH_2OH$	0.5	1	1	1	>100	>100	>100	1	50
8l	H	CH_2CONH_2	0.1	0.2	0.2	0.5	>100	>100	12.8	12.8	65
8m	H	$CH_2CONHCH_3$	1	2	1	2	>100	>100	12.8	12.8	55
8n	H	$CH_2CON(CH_3)_2$	4	8	8	16	>100	>100	12.8	12.8	150
8o	H		0.5	2	2	2	nt	nt	nt	nt	>100

Abbreviations: *C.a.*; *Candida albicans*, *C.t.*; *Candida tropicalis*, *A.f.*; *Aspergillus fumigatus*, *S.s.*; *Sporotrichum schenkii*, *T.m.*; *Trichophyton mentagrophytes*, *M.c.*; *Microsporum canis*.

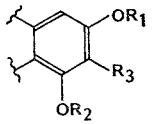
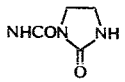
nt: Not tested.

Table 2. Antifungal activity *in vitro* (MIC) and *in vivo* (ED_{50}) of aminomethylene derivatives of **1**.

Compound				MIC (μg/ml)								ED_{50} (mg/kg, mice)
	R_1	R_2	R_3	*C.a.* K 1133	*C.a.* K 1082	*C.a.* K 75	*C.t.* ATCC 13803	*A.f.* ATCC 9197	*S.s.* ATCC 10212	*T.m.* ATCC 9533	*M.c.* ATCC 10214	*C.a.* K 1133 4×sc
9			H	>100	>100	>100	>100	>100	>100	12.5	>100	>300
10a	$CH_2N(CH_3)_2$	H	CH_3	12.5	12.5	12.5	25	>100	>100	>100	>100	>100
10b		H	CH_3	6.2	6.2	6.2	6.2	>100	>100	>100	3.1	200
11a		H	H	0.8	0.8	0.8	1.6	>100	>100	100	0.8	>100
11b		H	H	4	4	4	8	nt	nt	nt	nt	>100

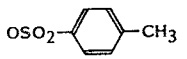
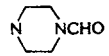
Abbreviations: See Table 1.
nt: Not tested.

Table 3. Antifungal activity *in vitro* (MIC) and *in vivo* (ED_{50}) of 11-aminopapulacandin derivatives.

Compound				MIC (μg/ml)								ED_{50} (mg/kg, mice)
				C.a. K 1133	*C.a.* K 1082	*C.a.* K 75	*C.t.* ATCC 13803	*A.f.* ATCC 9197	*S.s.* ATCC 10212	*T.m.* ATCC 9533	*M.c.* ATCC 10214	*C.a.* K 1133 4×sc
	R_1	R_2	R_3									
13a	CH_3	H	NH_2	0.5	1	1	2	>100	>100	12.8	12.8	>300
13c	H	CH_3	NH_2	0.5	1	1	2	nt	nt	nt	nt	nt
14a	CH_3	H	$NHCOCH_3$	2	4	2	4	nt	nt	nt	nt	90
15a	H	H	$NHCOCH_3$	0.2	1	0.5	1	>100	>100	12.8	>100	>100
15b	H	H	$NHSO_2CH_3$	0.1	0.5	0.5	0.5	nt	nt	nt	nt	30~50
15c	H	H		0.5	1	0.5	1	>100	>100	2	1	14

Abbreviations: See Table 1.
nt: Not tested.

Table 4. Antifungal activity *in vitro* (MIC) and *in vivo* (ED_{50}) of 6-substituted derivatives of **1**.

Compound	6-Substituent	MIC (μg/ml)								ED_{50} (mg/kg, mice)
		C.a. K 1133	*C.a.* K 1082	*C.a.* K 75	*C.t.* ATCC 13803	*A.f.* ATCC 9197	*S.s.* ATCC 10212	*T.m.* ATCC 9533	*M.c.* ATCC 10214	*C.a.* K 1133 4×sc
16		64	64	64	nt	nt	nt	nt	nt	nt
17a	N_3	0.1	0.2	0.2	0.5	>100	>100	12.8	12.8	>100
17b	Br	0.1	0.2	0.2	nt	>100	>100	12.8	12.8	>100
17c	I	0.1	0.5	0.2	0.5	>100	>100	12.8	12.8	>100
17d		2	4	2	4	nt	nt	nt	nt	>300
17e		1	2	2	2	nt	nt	nt	nt	55
17f		1	2	2	2	nt	nt	nt	nt	>100
17g		2	4	4	8	>100	>100	>100	8	>100
18a	$OCOCH_3$	0.5	1	1	2	>100	>100	12.8	12.8	>300
18b	$OCOCH_2CH_2COOH$	0.5	1	1	2	nt	nt	nt	nt	220

Abbreviations: See Table 1.
nt: Not tested.

shown). The removal of the short fatty acid by partial hydrolysis or the loss of the galactose moiety together with the short fatty acid (papulacandin D (**4**)) already leads to an almost complete loss of *in vitro* biological activity (data not shown)[2].

In the series of 12-mono- and 10,12-diether derivatives (compounds **5a**~**5h** and **6a**~**6h**) alkyl substituents lead with progressively increasing size to losses of activity (Table 1). More suitable for chemical derivatization is the phenolic hydroxyl group in position 10. The 10-alkyl ether derivatives **8a**, **8f**~**8o** generally exhibit good activity *in vitro*. Some compounds, including derivatives with polar residues (compounds **8f**~**8h**, **8k**~**8m**) are equally or even more active *in vivo* than papulacandin B (**1**): the oxypropyl ether **8f** is three times as active as **1**.

The 11,13-dimorpholinomethylene compound **9** and the 11-aminomethylene derivatives **10a**, **10b** and **11a**, **11b** (with free phenolic hydroxyl groups) are less active *in vitro* and *in vivo* than **1** (Table 2). Some of the derivatives in which the amino group is located directly on the aromatic ring, above all those with free phenolic hydroxyl groups, display notably improved *in vivo* activity by comparison with **1**. Besides showing good activity *in vitro*, the mesylate **15b**, for instance, and in particular the imidazolidone compound **15c** are much more active *in vivo* than papulacandin B (**1**) (Table 3).

While the substitution of the tosylate in **16** does produce derivatives with good activity *in vitro*, only the *N*-methylpiperazinyl compound **17e** is comparable with papulacandin B (**1**) in its activity *in vivo* (Table 4).

Of the two 6-acyl derivatives **18a** and **18b**, only the succinate **18b** still displays a weak effect *in vivo*, despite its having better water-solubility than **1** (Table 4).

Discussion

Examination of the biological data on the natural and degradation products of papulacandin B (**1**) and its derivatives reveals indications of the following structure-activity relations:

The presence of both fatty acids in unsaturated form and also of the galactosyl residue is essential to the biological activity of the substance. Even the removal of the short fatty acid or the absence of the galactose moiety together with the short fatty acid in papulacandin D (**4**)[2] results in almost complete loss of activity.

Not necessarily indispensable to the retention of biological activity, on the other hand, is the intact spirocyclic 5-ring. Chaetiacandin, in which the spirocycle is opened, has a similar spectrum of activity to that of papulacandin B (**1**)[6].

Of the two phenolic hydroxyl groups in the papulacandins, the one in position 10 is better suited for chemical modification than the one in position 12. Whereas in the 12-*O*-alkyl derivatives increasing size of the alkyl substituent rapidly leads to a progressive loss of activity, some 10-*O*-alkyl derivatives, including those with polar substituents, display slightly improved activity *in vivo* by comparison with **1**. The introduction of an aminomethylene group in position 11 of the aromatic ring tends to lead to a loss of activity; but some 11-aminoacyl compounds have notably better effects *in vivo* than **1**. The imidazolidone **15c** was found to be five times more active.

Positition 6 of the glucose residue proved relatively unsuitable for chemical derivatization.

None of all the papulacandin derivatives synthesized possessed a wider spectrum of activity including fungi other than *Candida albicans*.

The results of tests of the inhibitory action of these papulacandin derivatives on glucan biosynthesis in spheroplasts of *Candida albicans*[15] and the structure-activity relations inferred from these data are largely in agreement with the observations made *in vitro*. The sole exceptions were the hydrogenation products of papulacandin B and papulacandin D (**4**) which were only marginally active *in vitro*, but still exerted a good inhibitory effect on glucan biosynthesis[15]. On the basis of the foregoing observations, it can be concluded that the structural elements to which these compounds owe their

inhibitory action on glucan biosynthesis are quite distinct from those needed to ensure that they reach the target site, upon which ultimately depends their efficacy in inhibiting fungal growth.

Experimental

General

IR spectra were determined with a Perkin-Elmer 221 spectrophotometer. 100 MHz ^{1}H NMR spectra were recorded with a Varian XL-100-12, HA-100 or HA-100-D spectrometer and 360 MHz ^{1}H NMR spectra on a Bruker Spectrospin HX-360 spectrometer. ^{13}C NMR spectra were recorded on a Varian XL-100-15 spectrometer. Chemical shifts are expressed in ppm in relation to TMS as internal standard. Fast atom bombardment mass spectra (FAB-MS) were recorded with a ZAB HF spectrometer (VG-Manchester). For column chromatography Silica gel Merck 60 (0.063×0.20 mm) or Sephadex LH-20 (Pharmacia) was used. For TLC Silica gel plates F_{254} (Merck) were used.

Antibiotic Susceptibility

All *in vitro* antifungal activities are given as MIC in μg/ml. MICs were determined by the agar incorporation method. The ED_{50} value (dose protecting 50% of animals from death after intravenous infection) were determined according to the method described previously[16].

Ethers

Papulacandin B-12-methyl Ether (5a) and Papulacandin B-10,12-dimethyl Ether (6a)†

1 g Papulacandin B (**1**) was methylated with 2.86 g (10 equiv) silver oxide and 4.33 g (10 equiv) methyliodide in 100 ml DMF for 50 minutes at room temp until **1** could no longer be detected by TLC. The solution was filtered over Celite, evaporated to dryness *in vacuo* and chromatographed on silica gel. After precipitation from acetone - ether - hexane **5a** (0.3 g) and **6a** (0.4 g) were obtained as colorless amorphous powder.

5a: ^{1}H NMR (CD_3OD) δ 6.29 and 6.34 (2H, aromatic, H-11 and H-13), 3.80 (3H, s, aromatic, OCH_3); ^{13}C NMR (see Table 5); FAB-MS *m/z* 915 $(M+H)^+$, corresponding to $C_{48}H_{66}O_{17}$.

6a: ^{1}H NMR (CD_3OD) δ 6.48 (2H, aromatic, H-11 and H-13), 3.82 (6H, s, 2×aromatic, OCH_3); ^{13}C NMR (see Table 5); FAB-MS *m/z* 929 $(M+H)^+$, corresponding to $C_{49}H_{68}O_{17}$.

Papulacandin B-12-*p*-nitrobenzyl Ether (5e) and Papulacandin B-10,12-di-*p*-nitrobenzyl Ether (6e)

10 g **1**, dissolved in 300 ml DMF, was alkylated with 2.6 g (1 equiv) silver oxide and 4.32 g (1.8 equiv) *p*-nitrobenzyl bromide for 16 hours at room temp. After filtration of the solution over Celite, evaporation to dryness and chromatography on silica gel **5e** (4.3 g) and **6e** (4.9 g) were obtained as amorphous, pale yellow powder.

5e: ^{1}H NMR (CD_3OD) δ 7.75 and 8.20 (4H, aromatic); ^{13}C NMR (see Table 5).

Anal Calcd for $C_{54}H_{69}NO_{19}$: C 62.59, H 6.71, N 1.35.
Found: C 61.60, H 6.68, N 1.43.

6e: ^{1}H NMR (CD_3OD) δ 7.5~8.2 (8H, aromatic, overlapped by other signals); ^{13}C NMR (see Table 5).

Anal Calcd for $C_{61}H_{74}N_2O_{21}$: C 62.55, H 6.37, N 2.39.
Found: C 61.92, H 6.47, N 2.38.

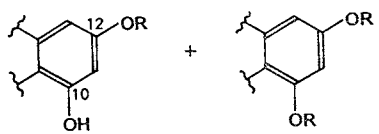
The 12-monoalkyl ethers **5b**~**5d**, **5f**~**5h** and the 10,12-dialkyl ethers **6c**, **6d**, **6f**~**6h** were prepared similarly to the preparation of **5a** and **6a**, or **5e** and **6e** using 5~10 equiv silver oxide and 5~20 equiv of either the corresponding alkyl iodide or alkyl bromide for alkylation. In the cases of **5g** and **6g** bromoacetic acid *p*-nitrobenzyl ester was used as alkylating agent. Removal of the protecting group, see under general procedure. For the ^{13}C NMR data of **5b**~**5h** and **6c**~**6h** see Table 5.

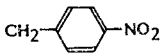
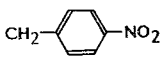
Papulacandin B-10-methyl Ether (8a)

3 g Papulacandin B-12-*p*-nitrobenzyl ether (**5e**) was methylated with 6.72 g (10 equiv) silver oxide and excess (30 ml) CH_3I in 250 ml DMF for 2.5 hours at 0°C to yield after filtration over Celite, evapora-

† The preparation of **5a** and **6a** from **1** with diazomethane has already been described[2].

Table 5. Selected ^{13}C NMR data of 12-alkyl ethers and 10,12-dialkyl ethers of **1** (all spectra in CD_3OD, δ ppm).



Compound	R	C-1	C-1′	C-6	C-6′	C-8	C-9	C-10	C-11/C-13	C-12	R
1	H	111.8	105.3	61.5	64.6	145.4	116.4	161.5	100.1/103.1	154.4	—
5a	CH_3	112.0	105.4	61.7	64.7	145.5	117.4	162.1	99.2/100.0	157.4	55.9
5b	CH_2CH_3	111.8	105.3	61.6	64.9	145.2	117.4	161.8	100.0/100.3	156.5	71.7/15.1
5c	$CH_2CH_2CH_3$	112.1	105.5	61.8	64.7	145.3	117.7	162.0	99.9/100.3	156.9	72.0/23.6/11.2
5d	$CH_2CH_2CH_2I$	111.7	105.3	61.6	64.5	145.2	117.6	161.8	100.0/100.3	156.1	74.0/34.2/2.7
5e		112.0	105.3	61.3	64.6	145.4	117.9	161.9	100.5/101.0	155.8	148.7/146.2/128.8/124.6/69.8
5f	CH_2COCH_3	111.8	105.2	61.5	64.6	145.6	117.7	161.8	100.3/101.4	155.3	207.3/76.1/27.0
5g	CH_2COOH	112.3	105.5	61.1	64.7	145.7	117.4	162.1	100.4/101.1	156.2	177.0/68.5
5h	CH_2COOCH_3	111.9	105.4	61.6	64.7	145.8	118.0	161.9	100.6/101.7	155.6	171.3/66.6/52.8
6a	CH_3	112.1	105.4	61.7	64.7	145.5	118.8	164.7	98.1/99.0	157.3	56.1 (2C)
6c	$CH_2CH_2CH_2$	112.0	105.4	61.7	64.7	145.3	118.8	163.9	98.6/100.2	156.7	71.9/71.1/23.6 (2C)/11.2/10.8
6d	$CH_2CH_2CH_2I$	111.9	105.4	61.7	64.6	145.3	119.3	163.4	99.1/100.3	156.1	78.4/77.4/34.3/34.0/2.7/2.5
6e		112.2	105.3	62.1	64.5	145.9	120.7	163.2	101.0/101.9	156.1	149.2/146.0/129.7/124.7/70.2
6f	CH_2COCH_3	111.9	105.2	61.5	64.7	145.8	119.8	162.5	99.9/101.8	155.4	207.1/206.7/76.7/74.0/26.4 (2C)
6g	CH_2COOH	112.5	105.6	61 *ca.*	64 *ca.*	145.7	118.8	163.6	100.0/100.0	156.0	176.9/176.6/69.3/68.9
6h	CH_2COOCH_3	111.9	105.4	61.5	64.7	145.8	120.3	162.5	100.3/101.2	155.5	171.1 (2C)/66.7/66.3/52.9/52.7

tion and chromatography papulacandin B-10-methyl-12-*p*-nitrobenzyl ether (**7a**) (1.6 g) as pale yellow powder.

Removal of the *p*-Nitrobenzyl-protecting Group (General Procedure)

1.6 g **7a** was stirred intensively for 15 minutes at 0°C with 3.2 g zinc dust in 100 ml acetic acid - methanol (7:3). The solution was filtered over Celite, evaporated to dryness and chromatographed on silica gel. After precipitation from acetone - ether - hexane the 10-methyl ether **8a** (0.65 g) was obtained as colorless amorphous powder.

^{1}H NMR (CD_3OD) δ 3.72 (3H, s, aromatic OCH_3); ^{13}C NMR (see Table 6); FAB-MS *m/z* 915 $(M+H)^+$, corresponding to $C_{48}H_{66}O_{17}$.

Papulacandin B-10-alkyl Ethers (**8f**~**8o**)

The 10-alkyl ethers **8f**~**8o** were prepared by alkylation of **5e** with silver oxide (5~10 equiv) and the corresponding alkyl bromide or alkyl iodide following deprotection by zinc dust in acetic acid - methanol as described in the general procedure. For the preparation of the acid **8g** bromoacetic acid *p*-nitrobenzyl ester was used as alkylating agent. ^{13}C NMR data of **8f**~**8o** see Table 6.

Aminomethylene Derivatives of Papulacandin B (**1**)

11,13-Dimorpholinomethylenepapulacandin B (**9**)

1 g **1** was dissolved in 100 ml of a methanolic solution containing 0.5 ml morpholine and 5 ml aqueous formaldehyde. After 1 hour at room temp, the solution was evaporated to dryness and the residue chromatographed on silica gel to afford **9** (0.75 g) as a pale yellow amorphous powder after precipitation from acetone - ether - hexane.

^{1}H NMR (CD_3OD) δ aromatic H-11- and H-13-protons missing, 2.8~3.4 (*ca.* 20H, overlapped by other signals); FAB-MS *m/z* 1,099 $(M+H)^+$, corresponding to $C_{57}H_{82}N_2O_{19}$.

11-Dimethylaminomethylenepapulacandin B-12-methyl Ether (**10a**)

0.7 g **5a**, dissolved in 150 ml methylene chloride, was strirred with 0.72 g (5 equiv) dimethylaminomethylene iodide for 2.5 hours at room temp. The solution was evaporated to dryness and chromatographed on Sephadex LH-20 affording 0.4 g **10a** after lyophilization.

^{1}H NMR (CD_3OD) δ 3.8 (3H, s, OCH_3), 2.6 (6H, s, $N(CH_3)_2$).

11-Morpholinomethylenepapulacandin B-12-methyl Ether (**10b**)

Reaction of 1.1 g papulacandin B-12-methyl ether (**5a**) with 0.53 ml morpholine and 5.5 ml formaldehyde in 110 ml methanol (24 hours at room temp) as described for the preparation of **9** afforded **10b** (0.75 g) as colorless amorphous powder.

^{1}H NMR (CD_3OD) δ 3.78 (3H, s, aromatic, OCH_3), *ca.* 3.7 (4H, 2×OCH_2), *ca.* 2.1~2.5 (4H, 2×NCH_2); ^{13}C NMR (CD_3OD) δ 161.5 (C-10), 156.6 (C-12), 143.8 (C-8), 117.1 (C-9), 107.4 (C-11), 99.9 (C-13), 67.8 (2C, OCH_2), 57.4 (2C, CH_2N), 56.1 (aromatic, OCH_3), 54.0 (NCH_2); FAB-MS *m/z* 1,014 $(M+H)^+$, corresponding to $C_{53}H_{75}NO_{18}$.

Compounds **10c** and **10d** were prepared as described for the preparation of **9** starting from papulacandin B-12-*p*-nitrobenzyl ether (**5e**) by reaction with formaldehyde and the corresponding secondary amine. Removal of the *p*-nitrobenzyl-protecting group from **10c** and **10d** by the general procedure described above gave the 11-aminomethylenepapulacandin B derivatives **11a** and **11b**.

11-Morpholinomethylenepapulacandin B (**11a**)

^{13}C NMR (CD_3OD) δ 143.6 (C-8), 111.0 (C-1), 105.4 (C-1'), 67.8 and 67.6 (2×OCH_2), 64.9 (C-6'), 61.6 (C-6), 54.3 and 54.2 (NCH_2).

11-Piperidinomethylenepapulacandin B (**11b**)

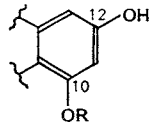
^{13}C NMR (CD_3OD) δ 161.9 (C-10), 154.3 (C-12), 143.8 (C-8), 115.9 (C-9), 105.8 (C-11), 103.8 (C-13), 64.7 (C-6'), 61.5 (C-6), 57.9 (CH_2N), 54.5 (2C, NCH_2), 26.2 (2C) and 24.4 (3×CH_2).

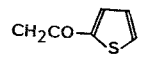
11-Aminopapulacandin B Derivatives

11-Aminopapulacandin B-12-methyl Ether (**13a**)

To a solution of 6 g papulacandin B-12-methyl ether (**5a**) in 250 ml THF were added 12.71 g (12

Table 6. Selected ^{13}C NMR data of 10-alkyl ethers of papulacandin B (**1**) (all spectra in CD_3OD, δ ppm).



Compound	R	C-1	C-1′	C-6	C-6′	C-8	C-9	C-10	C-11	C-12	C-13	R
8a	CH_3	112.0	105.4	61.6	64.9	145.6	117.8	164.3	58.3	154.6	102.3	56.0
8f	CH_2COCH_3	112.0	105.4	61.6	64.8	145.8	118.6	162.4	59.0	154.7	103.0	207.0/75.0/26.3
8g	CH_2COOH	112.0	105.3	61.4	64.8	145.5	118.2	162.8	99.1	154.5	103.0	185.1/74.9
8h	CH_2COOCH_3	111.8	105.2	61.4	64.6	145.5	118.4	162.1	99.0	154.5	103.0	171.1/66.1/52.6
8i	$CH_2COOCH_2CH_3$	111.7	105.1	61.4	64.6	145.4	118.4	162.0	99.0	154.3	102.9	170.5/66.2/62.2/14.4
8k	$CH_2COOCH_2CH(OH)CH_2OH$	111.7	104.8	61.6	64.4	145.0	118.7	161.8	99.1	154.2	103.0	169.5/66.8/66.1/63.9
8l	CH_2CONH_2	112.0	105.4	61.6	64.9	145.8	118.9	162.1	99.5	154.7	102.9	173.9/68.2
8m	$CH_2CONHCH_3$	112.0	105.4	61.6	64.8	145.8	119.2	162.0	99.5	154.7	102.9	171.5/68.6/26.1
8n	$CH_2CON(CH_3)_2$	111.8	105.2	61.4	64.7	146.0	118.3	162.2	99.0	154.3	103.1	170.5/67.4/36.6/35.9
8o		111.8	105.3	61.5	64.7	145.5	118.5	162.2	99.2	154.5	103.1	189.9/141.0/136.1/ 134.7/129.7/70.1

equiv) hexamethyldisilazane and 9.46 g (4 equiv) diphenylseleno anhydride. The deep red reaction mixture was stirred at room temp for 7 minutes, water added and extracted three times with ethyl acetate. The combined ethyl acetate extracts were dried, evaporated to dryness and chromatographed on silica gel to afford 2.3 g of the phenylselenoimine **12a** as a deep red powder, which was reduced to **13a** by bubbling H_2S through a solution of **12a** in $CHCl_3$ and triethylamine for 5 minutes at room temp. Chromatography of the crude product on silica gel afforded 11-aminopapulacandin B-12-methyl ether (**13a**) as an amorphous, colorless powder (1.25 g). FAB-MS *m/z* 930 $(M+H)^+$, corresponding to $C_{48}H_{67}NO_{17}$; ^{13}C NMR (see Table 7).

11-Aminopapulacandin B-12-*p*-nitrobenzyl Ether (**13b**)

13b was obtained from 4 g papulacandin B-12-*p*-nitrobenzyl ether (**5e**), 5.4 g (4 equiv) diphenylseleno anhydride and 3.1 g (4 equiv) hexamethyldisilazane in 100 ml THF (20 minutes at 5~20°C) following reduction with H_2S in $CHCl_3$ and triethylamine (5 minutes, room temp). The yield was 1 g. ^{13}C NMR (see Table 7).

11-Aminopapulacandin B-10-methyl Ether (**13c**)

13c was prepared similarly to the preparation of **13a** from papulacandin-10-methyl ether (**8a**). ^{13}C NMR (CD_3OD) δ 149.2 and 148.5 (C-10+C-12), 122.4 and 117.3 (C-9 and C-11), 112.2 (C-1), 105.3 (C-1'), 100.4 (C-13), 64.7 (C-6'), 61.6 (C-6), 56.6 (OCH_3).

11-Aminoacetylpapulacandin B-12-methyl Ether (**14a**)

130 mg 11-aminopapulacandin B-12-methyl ether (**13a**) was acetylated in 1 ml methanol and 1 ml acetic anhydride for 30 minutes at 0°C. The mixture was evaporated to dryness and chromatographed on preparative thin-layer plates to afford **14a** (85 mg). ^{13}C NMR (see Table 7); FAB-MS *m/z* 994 $(M+Na)^+$, corresponding to $C_{50}H_{69}NO_{18}$.

11-Aminoacetylpapulacandin B (**15a**)

250 mg **13b** was acetylated in methanol - acetic anhydride for 30 minutes at room temp. Reduction of the crude product (**14b**) in Zn - acetic acid (general procedure) and chromatography on preparative thin-layer plates afforded **15a**. FAB-MS *m/z* 980 $(M^+ + Na)^+$, corresponding to $C_{49}H_{67}NO_{18}$.

11-Mesylaminopapulacandin B (**15b**)

300 mg **13b** was mesylated with 47 mg mesyl chloride in 10 ml THF and 20 drops of pyridine at room temp overnight. Water was added and the solution extracted with ethyl acetate. The crude product **14c** was reduced with Zn in methanol - acetic acid (general procedure) and chromatographed on silica gel to yield 80 mg of the mesylamino derivative **15b**. ^{13}C NMR (see Table 7).

11-Amino-(1-carboxamido-2-oxoimidazolinyl)papulacandin B (**15c**)

1.2 g **13b** was acylated with 204 mg 2-oxoimidazolin-1-carboxylic acid chloride in 40 ml THF and 1 ml pyridine for 1.5 hours at 0°C. Water was added and the solution extracted with ethyl acetate. The crude product (**14d**) was reduced with Zn-powder (general procedure) to remove the protecting group. Chromatography on silica gel gave 0.45 g **15c**. ^{13}C NMR (see Table 7).

Derivatives in Position 6 of the Glucose-moiety

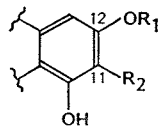
6-Tosylpapulacandin B (**16**)

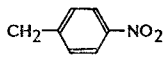
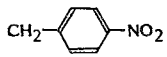
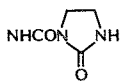
To 5 g **1** in 100 ml abs pyridine was added a solution of 6.35 g tosyl chloride (5 equiv) in 50 ml pyridine. After 1 hour at 0°C 500 g crushed ice was added and the aqueous solution extracted three times with ethyl acetate. The combined ethyl acetate extracts were washed several times with 1 N HCl, saturated sodium bicarbonate solution and H_2O. The organic phase was dried, evaporated to an oily residue which gave after chromatography and precipitation from acetone - ether - hexane 6-tosylpapulacandin B (**16**) as a pale yellow amorphous powder. ^1H NMR (CD_3OD) δ 7.15 and 7.68 (4H, aromatic), 2.35 (tosyl-CH_3); ^{13}C NMR (see Table 8).

6-Dehydroxy-6-azidopapulacandin B (**17a**)

0.5 g **16** and 0.3 g (5 equiv) NaN_3 in 25 ml DMF were heated under stirring to 80°C for 1 hour.

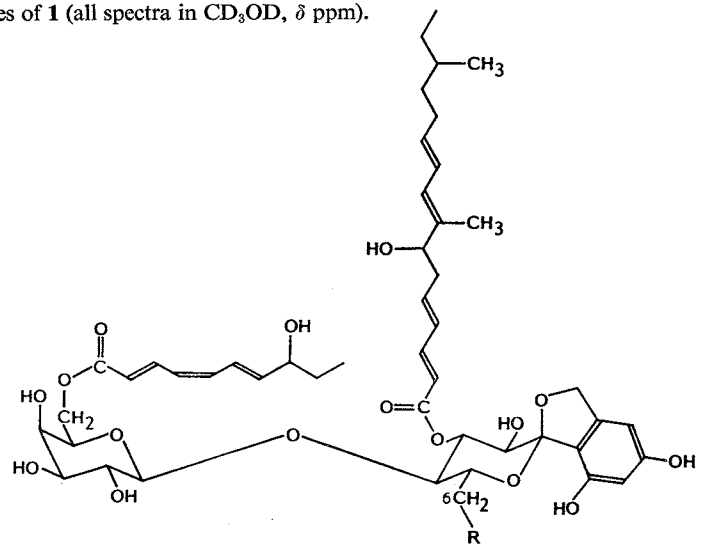
Table 7. Selected ^{13}C NMR data of 11-aminopapulacandin B derivatives (all spectra in CD_3OD, δ ppm).

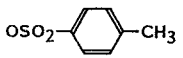
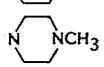
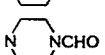
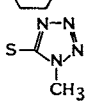


Compound	R_1	R_2	C-1	C-1′	C-6	C-6′	C-8	C-9/ C-11	C-10/ C-12	C-13	R_1	R_2
13a	CH_3	NH_2	112.2	105.2	61.6	64.6	(142.3)	122.4/ 117.2	149.2/ 148.5	100.3	56.5	—
13b		NH_2	112.5	105.5	61.4	64.7	142.4	121.1/ 118.5	148.5/ 148.5	102.2	149.0/146.9/ 129.1/124.7	—
14a	CH_3	$NHCOCH_3$	112.4	105.5	61.7	64.7	141.0	117.7/ 112.4	155.7/ 155.6	100.5	56.3	172.5/22.6
14b		$NHCOCH_3$	112.4	105.4	61.3	64.6	140.8	118.2/ 112.9	155.5/ 153.8	101.9	148.8/146.2/ 128.9/124.7	172.5/22.7
15b	H	$NHSO_2CH_3$	112.3	105.2	61.5	64.8	(140)	117.2/ 110.6	156.8/ 153.7	103.8	—	40.1
15c	H		112.1	105.2	61.5	64.8	140.3	116.7/ 111.6	154.4/ 153.9	104.3	—	160.2/43.3/ 37.6

The chemical shifts in parenthesis were not clearly assigned.

Table 8. Selected ^{13}C NMR data of 6-substituted derivatives of **1** (all spectra in CD_3OD, δ ppm).



Compound	R	C-1	C-1′	C-6	C-6′	C-8	C-9	C-10	C-11	C-12	C-13	R
1	H	111.8	105.3	61.5	64.6	145.4	116.4	161.5	100.1	154.4	103.1	—
16		111.4	105.2	69.9	64.5	145.2	115.9	161.3	99.6	154.9	102.8	145.9/133.9/130.7(2C)/ 129.1(2C)/21.6
17a	N_3	111.6	105.5	(51.8)	64.7	145.2	116.1	161.4	99.5	155.1	102.8	—
17b	Br	111.5	105.2	(48.4)	64.6	145.2	116.0	161.3	99.6	155.0	102.8	—
17c	I	111.4	105.3	8.3	64.6	145.2	116.0	161.2	99.6	154.9	102.7	—
17d		111.4	105.7	59.1	64.7	145.0	116.2	161.3	99.6	154.9	102.6	67.3(2C)/55.0(2C)
17e		111.5	105.6	58.1	64.5	145.0	116.4	161.4	99.5	155.0	102.8	55.2(2C)/53.4(2C)/45.2(NCH_3)
17f		111.6	105.6	58.2	64.7	145.2	116.0	161.6	99.6	155.1	102.8	163.2/55.3/54.1/41.7/40.9
17g		111.5	105.9	36.8	64.6	145.1	115.8	161.5	99.6	154.9	102.8	155.5/34.3(NCH_3)
18a	$OCOCH_3$	111.8	105.1	64.7	64.7	145.1	116.3	161.6	99.7	155.2	102.9	173.0/20.9
18b	$OCO(CH_2)_2COOH$	111.5	105.2	64.2	64.6	145.3	116.1	161.4	99.7	155.0	109.2	174.5/30~32(2C)

The chemical shifts in parentheses were overlapped by other signals.

Crushed ice was added to the reaction mixture and the solution extracted three times with ethyl acetate. The combined organic extracts were dried and evaporated, and the crude product chromatographed on silica gel to afford the azide **17a** (0.3 g) as pale yellow amorphous powder. IR (KBr) cm^{-1} 3500, 2970, 2220 (N_3, strong), 1700, 1615; ^{13}C NMR (see Table 8).

6-Dehydroxy-6-bromopapulacandin B (**17b**)

0.5 g **16** and 1.1 g (20 equiv) KBr in 25 ml DMF were heated under stirring to 100°C for 2.5 hours. After cooling crushed ice was added and the solution extracted with ethyl acetate. The combined ethyl acetate extracts were dried, evaporated and chromatographed on silica gel to afford **17b** (275 mg). FAB-MS *m/z* 963 $(M+H)^+$, corresponding to $C_{47}H_{63}BrO_{16}$; ^{13}C NMR (see Table 8).

6-Dehydoxy-6-iodopapulacandin B (**17c**)

17c was prepared from 0.5 g **16** and 1.42 g (20 equiv) NaI in 25 ml DMF as described for the preparation of **17b** (100°C for 1.5 hours). ^{13}C NMR (see Table 8).

6-Dehydroxy-6-morpholinopapulacandin B (**17d**)

325 mg **16** was refluxed with 20 ml ethanol containing 1 ml morpholine for 3 hours. The solution was concentrated *in vacuo* and extracted with ethyl acetate after addition of ice-water. Chromatography of the crude product on preparative thin-layer silica gel plates afforded **17d** (120 mg). ^{13}C NMR (see Table 8).

6-Dehydroxy-6-*N*-methylpiperazinylpapulacandin B (**17e**)

17e was prepared from 1 g **16** and 2 ml *N*-methylpiperazine in 40 ml DMF as described for the preparation of **17d** (60°C, 3 hours). ^{13}C NMR (see Table 8).

6-Dehydroxy-6-(*N*-formylpiperazinyl)papulacandin B (**17f**)

17f was prepared from 2 g **16** and 2 g *N*-formylpiperazine in 100 ml DMF as described for the preparation of **17d** (80°C, 7.5 hours). ^{13}C NMR (see Table 8).

6-Dehydroxy-6-(*N*-methylthiotetrazolinyl)papulacandin B (**17g**)

17g was prepared from 800 mg **16** and 800 mg 1-methyl-5-thiotetrazoline sodium salt in 40 ml DMF as described for the preparation of **17d** (80°C, 5 hours). ^{13}C NMR (see Table 8).

6-Acetylpapulacandin B (**18a**)

2.5 g papulacandin B-10,12-di-*p*-nitrobenzyl ether (**6e**) was persilylated in 50 ml abs pyridine with 15 ml hexamethyldisilazane and 10 ml TMS at room temp for 40 minutes. The solution was centrifuged, the supernatant evaporated *in vacuo* and the oily residue dried *in vacuo*. The residue was then dissolved in 20 ml abs pyridine - acetic acid anhydride (1 : 1). 0.75 ml (6 equiv) acetic acid was added and the solution left at room temp for 24 hours. Ice-water was added and the aqueous solution extracted several times with ethyl acetate. The combined ethyl acetate extracts were dried, evaporated to dryness and the oily residue dissolved in acetic acid - 50% methanol (1 : 1). The solution was kept at room temp for 5 hours, triturated with 1 N HCl to pH 7.2. Water was then added and the solution extracted three times with ethyl acetate. The combined extracts were dried, evaporated and chromatographed on silica gel. After precipitation from acetone - ether - hexane 6-acetylpapulacandin B-10,12-di-*p*-nitrobenzyl ether was obtained, which, after removal of the two protecting groups (general procedure), afforded 6-acetylpapulacandin B (**18a**) (175 mg). ^1H NMR (CD_3OD) δ 2.0 (3H, s, additional, $COCH_3$); ^{13}C NMR (see Table 8); FAB-MS *m/z* 943 $(M+H)^+$, corresponding to $C_{49}H_{66}O_{18}$.

6-Succinylpapulacandin B (**18b**)

18b was prepared similarly to **18a** from **6e** and succinic acid anhydride. Final purification was achieved by chromatography on Sephadex LH-20. ^{13}C NMR (see Table 8); FAB-MS *m/z* 1,001 $(M+H)^+$, corresponding to $C_{51}H_{68}O_{20}$.

Acknowledgment

We acknowledge with thanks the contribution of Mr. E. LACH for his careful technical assistance, Mr. S.

Moss for the IR and UV spectra, Mr. H. FUHRER for the NMR spectra, Mr. F. RASCHDORF for the MS spectra, Mr. W. PADOWETZ for the micro-analysis, Miss J. GYSIN for the MIC tests and Mr. R. FRISCHKNECHT and Mrs. F. SCHAEFFER for the *in vivo* tests. Thanks are also due to Mr. A. KIRKWOOD for reviewing the manuscript.

References

1) GRUNER, J. & P. TRAXLER: Papulacandins, a new antibiotic, active against yeasts. Experientia 33: 137, 1977
2) TRAXLER, P.; J. GRUNER & J. A. L. AUDEN: Papulacandins, a new family of antibiotics with antifungal activity. I. Fermentation, isolation, chemical and biological characterization of papulacandins A, B, C, D and E. J. Antibiotics 30: 289~296, 1977
3) TRAXLER, P.; H. FRITZ, H. FUHRER & W. J. RICHTER: Zur Struktur von Papulacandin B, einem neuen antifungischen Antibiotikum. Helv. Chim. Acta 60: 578~584, 1977
4) TRAXLER, P.; H. FRITZ, H. FUHRER & W. J. RICHTER: Papulacandins, a new family of antibiotics with antifungal activity. Structures of papulacandins A, B, C and D. J. Antibiotics 33: 967~978, 1980
5) RHIS, G. & P. TRAXLER: Röntgenstrukturanalyse eines neuartigen spirocyclischen Diglycosids. Helv. Chim. Acta 64: 1533~1539, 1981
6) KOMORI, T.; M. YAMASHITA, Y. TSURUMI & M. KOHSAKA: Chaetiacandin, a novel papulacandin. I. Fermentation, isolation and characterization. J. Antibiotics 38: 455~459, 1985
7) KOMORI, T. & Y. ITOH: Chaetiacandin, a novel papulacandin. II. Structure determination. J. Antibiotics 38: 544~546, 1985
8) BAGULEY, B. C.; G. ROEMMELE, J. GRUNER & W. WEHRLI: Papulacandin B: An inhibitor of glucan synthesis in yeast spheroplasts. Eur. J. Biochem. 97: 345~351, 1979
9) PÉREZ, P.; R. VARONA, I. GARCÍA-ACHA & A. DURÁN: Effect of papulacandin B and aculeacin A on β-(1–3)glucan-synthase from *Geotrichum lactis*. FEBS Lett. 129: 249~252, 1981
10) PÉREZ, P.; I. GARCÍA-ACHA & A. DURÁN: Effect of papulacandin B on the cell wall and growth of *Geotrichum lactis*. J. Gen. Microbiol. 129: 245~250, 1983
11) VARONA, R.; P. PÉREZ & A. DURÁN: Effect of papulacandin B on β-glucan synthesis in *Schizosaccharomyces pombe*. FEMS Microbiol. Lett. 20: 243~247, 1983
12) BARTON, D. H. R.; A. G. BREWSTER, S. V. LEY & M. N. ROSENFELD: Preparation of phenylselenoimines from phenols using diphenylselenic anhydride and hexamethyldisilazane. J. Chem. Soc. Chem. Commun. 1977: 147~148, 1977
13) MCINNES, A. G.: The alcoholysis of trialkylalkoxysilanes. Can. J. Chem. 43: 1998, 1965
14) FUCHS, E. F. & J. LEHMANN: Notiz über eine verbesserte Methode zur spezifischen Freisetzung oder Acylierung primärer Hydroxylgruppen ausgehend von pertrimethylsilylierter Polyolen. Chem. Ber. 107: 721~724, 1974
15) RÖMMELE, G.; P. TRAXLER & W. WEHRLI: Papulacandins—The relationship between chemical structure and effect on glucan synthesis in yeast. J. Antibiotics 36: 1539~1542, 1983
16) ZAK, O.: Usefulness and limitations of animal models in the study of opportunistic nonbacterial infections. *In* Infections in Cancer Patients. *Ed.*, J. KLASTERSKY, pp. 25~48, Raven Press, New York, 1982