PAPULACANDINS, A NEW FAMILY OF ANTIBIOTICS WITH ANTIFUNGAL ACTIVITY

STRUCTURES OF PAPULACANDINS A, B, C AND D

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The structures of the papulacandins A, B, C and D, new antibiotics of *Papularia sphaerosperma* have been established by means of spectral analysis and degradation reactions. Base catalysed hydrolysis of the main product papulacandin B (**1**) gave two new hydroxylated long-chain unsaturated fatty acids **5** and **6** along with a hitherto unknown spirocyclic diglycoside **7**. The structure of **7** was determined by further degradation reactions. The positions of attachment of the two fatty acids to the spirocyclic diglycoside **7** through ester-bonds were established by selective base catalysed hydrolysis of **1** and spectral analysis of **1** and some derivatives and degradation products thereof. The structures of papulacandin A (**2**), papulacandin C (**3**) and papulacandin D (**4**) were determined in an analogous way.

In the course of our screening for new antibiotics we found that a strain of *Papularia sphaerosperma* produces a series of new antifungal antibiotics[1]. Five structurally closely related compounds, namely papulacandins A, B, C, D, and E were isolated[2]. They all show strong activity against *Candida albicans* and several other yeasts but have little or no activity against other fungi, bacteria or protozoa. Papulacandin B was shown to be an inhibitor of glucan synthesis in yeast spheroplasts and differs therein in its mode of action from the polyene antibiotics[3].

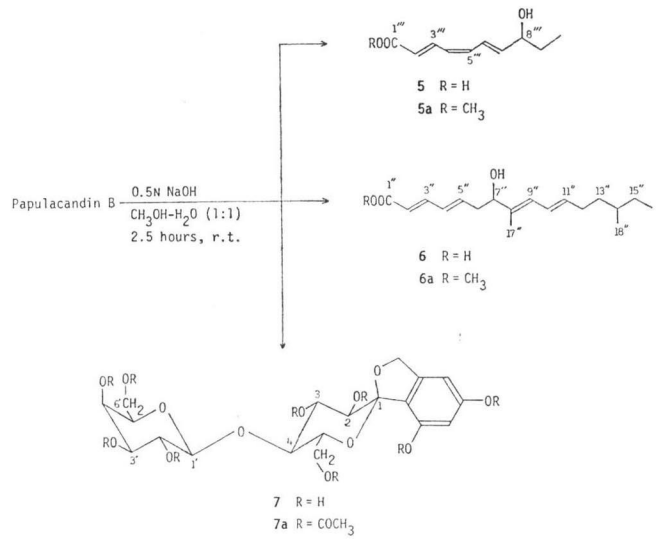
In a preliminary communication the structure of the main component papulacandin B was shown to consist of a spirocyclic diglycoside with two hydroxyl groups esterified by two hydroxylated long-chain unsaturated fatty acids[4]. In the present account details of the structure elucidation of this antibiotic as well as the structures of several congeners, *i.e.* papulacandins A, C, and D, will be discussed.

Structure of Papulacandin B (1)

Structure **1** has been established for papulacandin B ($C_{47}H_{84}O_{17}$) by means of spectral analysis of the intact antibiotic and some of its derivatives and degradation products[4]. Base catalysed hydrolysis of **1** gave the two unsaturated fatty acids **5** and **6** together with the spirocyclic diglycoside **7** (Scheme 1). To our knowledge the structures of these constituents have not yet been described in the literature.

The first of the two fatty acids **5** is a triply unsaturated, fully conjugated C_{10}-hydroxycarboxylic acid. Its structure was determined on the basis of the spectroscopic data of its methyl ester **5a** ($C_{11}H_{16}O_3$, $M^{+\cdot}$ at *m/z* 196), using mainly results from 360-MHz-^{1}H-NMR-(with decoupling experiments), the ^{13}C-NMR- (Table 2) and the EI low and high resolution mass spectra. The coupling constants in the ^{1}H-NMR-spectrum indicate a *trans-cis-trans* configuration of the three conjugated double bonds. The structural determination of the second fatty acid **6** was also based on spectral analyses of its methyl ester **6a** ($C_{19}H_{30}O_3$, $M^{+\cdot}$ at *m/z* 306). In both the 360-MHz-^{1}H-NMR- and the ^{13}C-NMR-spectrum all

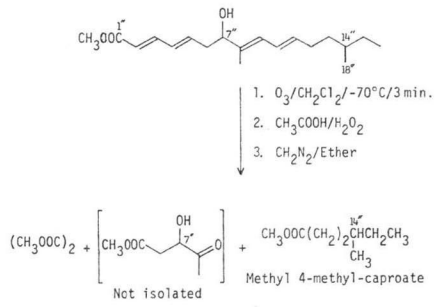
Scheme 1. Base catalysed hydrolysis of papulacandin B (**1**).



proton and carbon signals could be assigned (experimental part and Table 2). In addition, most of the coupling protons could be connected in the ^{1}H-NMR-spectrum by double resonance experiments.

Further evidence for the postulated structure of the fatty acid **6**, especially with respect to the position of the methyl group at C (14″), was obtained by an ozonolytic cleavage (Scheme 2). Ozonolysis of the methyl ester **6a** followed by oxidation with H_2O_2 in acetic acid and esterification with CH_2N_2 gave dimethyl oxalate and methyl 4-methylcaproate. Gas chromatography showed the latter to be identical with authentic material (racemic sample). These results establish the structure of **6** as that of a 7-hydroxy-8,14-dimethyl-2,4,8,10-hexadecatetraenoic acid (**6**) with all-*trans* stereochemistry of the double bonds, but unknown configuration of C (7″) and C(14″).

Scheme 2. Ozonolytic cleavage of methyl 7-hydroxy-8,14-dimethyl-2,4,8,10-hexadecatetraenoate (**6a**).



The structure of the third product of the alkaline hydrolysis, the spirocyclic diglycoside **7** ($C_{19}H_{26}O_{13}$, $M^{+\cdot}$ of its nonaacetate $C_{37}H_{44}O_{22}$ at *m/z* 840) was determined by further degradation reactions (Scheme 3): methylation of the two phenolic hydroxyl groups with CH_3I in the presence of Ag_2O gave the dimethyl ether **8** ($C_{21}H_{30}O_{13}$: $M^{+\cdot}$ of its heptaacetate $C_{35}H_{44}O_{20}$ at *m/z* 784). Methanolysis of **8** with CH_3OH - HCl gave two main products, α-D-methyl-galactopyranoside (**9**), identified as its tetraacetate **9a** by comparison with an authentic sample and compound **10** ($C_{15}H_{20}O_8$, $M^{+\cdot}$ at *m/z* 328), which was oxidised with periodic acid to give 5,7-dimethoxyphthalide (**11**) ($C_{10}H_{10}O_4$, $M^{+\cdot}$ at *m/z* 194), whose spectroscopic data were in agreement with data given in the literature[5].

In addition, an X-ray crystallographic analysis of the 10,12-di-*p*-bromobenzyl ether of the diglycoside **7** showed the second sugar unit to be a glucose, and the galactose to be linked with the glucose *via* a β-*trans*-1,4-linkage[6]. Concerning the stereochemistry at C (1), the oxygen atom of the spirocyclic

Table 1. Selected NMR-data of papulacandins A, B, C and D, tetradecahydropapulacandin B, its nonaacetate and some degradation products of **1**. (δ-values in ppm; coupling constant J in Hz in brackets)

Compound	H–2	H–3	H–7	H–11+13	H–1′	H–2′	H–3′	H–5′	H–6′
Papulacandin A (**2**)	4.33d (10)	5.38t (9)	5.00AB (12)	6.16s 6.17s	~4.3[a]	~3.5[a]	~3.5[a]	3.66t (6)	4.13dd (6/11) 4.21dd (6/11)
Papulacandin B (**1**)	4.32d (10)	5.44t (10)	5.02AB (12)	6.21s 6.24s	~4.3[a]	~3.5[a]	~3.5[a]	3.65t (5.5)	4.11dd (7/11) 4.25dd (5.5/11)
Tetradecahydro-pap. B	4.33d (10)	5.36t (10)	5.03AB (12)	6.19s 6.22s	4.30d (8)	~3.5[a]	~3.5[a]	3.65t (5)	4.20dd (5/11) 4.25dd (7/11)
Tetradecahydro-pap. B nonaacetate	5.61d (10)	5.55t (10)	5.13AB (12)	6.87s 6.99d	4.45d (8)	5.10dd (8/10)	4.90dd (4/10)	a)	a)
Papulacandin D	4.34d (10)	5.34t (10)	5.03AB (12)	6.19s 6.20s	—	—	—	—	—
12	4.33d (10)	5.40t (10)	4.97AB (12)	6.19s 6.20s	4.28d (8)	3.5dd[b] (8/10)	3.5dd[b] (8/10)	shifted to higher field[a]	
13	5.68d (10)	4.07t (10)	5.00AB (12)	6.14s 6.16s	4.45d (8)	3.56dd (8/10)	3.49dd (3/10)	shifted to higher field[a]	
7a	5.75d (10)	5.66dd (10/10)	5.26s	6.91s 6.98s	4.60d (8)	5.25dd (8/10)	5.06dd (4/10)	4.01m (8/10)	~4.15[a]
10	4.17d (10)	3.69t (10)	—	—	—	—	—	—	—
10a	5.81d (10)	5.47t (10)	—	—	—	—	—	—	—
β-D-O-Methylgalacto-pyranosidetetraacetate[7]	—	—	—	—	4.45dd (1.5/7.5)	5.21dd (7.5/10.5)	5.06dd (3/10.5)	3.98m[b] (1.5/6.5)	4.19AB[b]

a) No exact location possible because of overlapping of other signals.
b) Signal is overlapped by other signals.

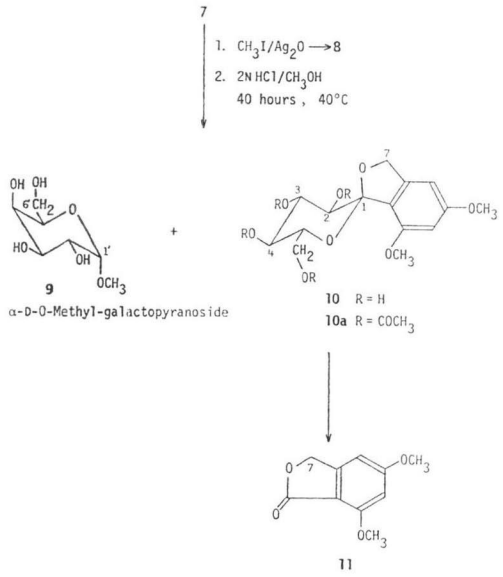
Table 2. Tentative assignment of the carbons in the ^{13}C-NMR-spectra of papulacandins A, B, C and D and some derivatives and degradation products of papulacandins A and B.
(δ-values in ppm)

Carbon	Pap. A (CD_3OD)	Pap. B (CD_3OD)	Pap. C (CD_3OD)	Pap. D (CD_3OD)	**12** (CD_3OD)	**13** (CD_3OD)	**7** (CD_3OD)	**10** ($CDCl_3$)	**5a** ($CDCl_3$)	**6a** ($CDCl_3$)	**14** ($CDCl_3$)
1	111.8	111.9	112.0	111.9	111.9	110.5	111.9	112.0	—	—	—
1′	105.3	105.3	105.3	—	105.3	104.8	105.0	—	—	—	—
2	77.8	77.6	77.8	78.2	77.4	79.5	80.5	76.3	—	—	—
3	77.5	77.6	77.7	75.5	77.4	77.4	77.0	75.8	—	—	—
4	77.5	74.7	74.8	73.7	76.5	77.4	74.8	73.5	—	—	—
5	74.6	74.1	74.8	71.7	74.8	74.7	74.8	73.4	—	—	—
2′ a)	74.6	74.0	74.3	—	74.8	74.7	74.3	—	—	—	—
3′	73.8	74.0	74.0	—	73.9	74.3	73.8	—	—	—	—
4′	72.5	72.6	72.7	—	72.8	73.0	73.2	—	—	—	—
5′	71.8	71.8	72.0	—	71.8	72.6	72.6	—	—	—	—
7	70.1	70.3	70.3	69.5	69.9	70.5	70.3	72.0	—	—	—
6	61.5	61.6	61.6	62.2	61.6	61.4	62.0	63.0	—	—	—
6′	64.6	64.9	64.7	—	62.0	62.7	62.6	—	—	—	—
8	145.4	145.5	145.6	145.3	145.4	144.9	145.5	145.4	—	—	—
9	116.4	116.5	116.6	116.4	116.4	115.2	116.8	119.1	—	—	—
10	161.5	161.6	161.7	161.3	161.5	161.8	161.4	164.3	—	—	—
11	100.1	100.0	100.2	100.0	100.1	100.0	100.2	97.9	—	—	—
12	154.4	154.5	154.6	154.4	154.4	154.7	154.5	157.2	—	—	—
13	103.1	103.0	103.2	103.0	103.1	103.4	103.2	98.9	—	—	—
1″	169.0	169.1	169.2	169.0	169.1	167.7	—	—	—	167.7	—
2″	121.5	121.6	121.3	120.7	121.5	120.1	—	—	—	119.6	—
3″	146.0	146.1	146.1	146.4	146.2	146.5	—	—	—	144.9	—
4″ a)	127.1	127.1	127.2	126.9	127.1	127.0	—	—	—	126.2	—
10″ a)	126.9	127.1	127.2	126.8	126.9	126.9	—	—	—	125.5	—

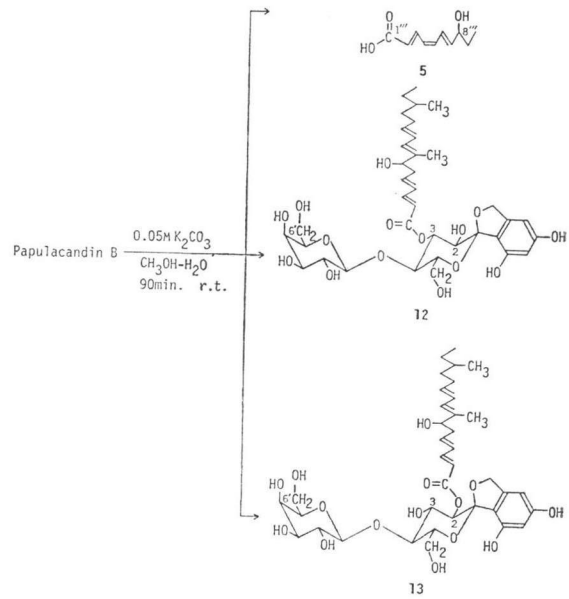
5″ a)	137.6	137.5	137.7	137.4	137.6	137.5	—	—	—	136.1	—
8″ a)	136.0	136.1	136.3	136.0	136.1	136.1	—	—	—	136.1	—
9″	131.5	131.5	131.6	131.3	131.5	131.3	—	—	—	130.6	—
11″	141.7	141.6	141.7	141.8	141.8	142.1	—	—	—	140.1	—
7″	76.3	76.4	76.5	77.3	76.2	77.1	—	—	—	76.5	—
6″	40.1	40.0	40.1	39.8	40.4	39.9	—	—	—	38.9	—
12″	31.5	31.6	31.6	31.4	31.5	31.5	—	—	—	30.6	—
13″	37.5	37.5	37.6	37.3	37.3	37.4	—	—	—	36.3	—
14″	35.1	35.2	35.3	35.0	35.1	35.1	—	—	—	34.0	—
15″	30.4	30.4	30.5	30.3	30.4	30.4	—	—	—	29.4	—
16″	11.7	11.7	11.7	11.6	11.7	11.7	—	—	—	11.3	—
17″	19.5	19.5	19.5	19.4	19.5	19.5	—	—	—	19.1	—
18″	12.3	12.2	12.3	12.2	12.2	12.3	—	—	—	12.2	—
1‴	168.5	168.4	168.5	—	—	—	—	—	168.5	—	173.0
2‴	121.7	122.1	121.7	—	—	—	—	—	121.9	—	120.4
3‴	143.2	143.5	146.2	—	—	—	—	—	143.1	—	143.1
4‴	127.5	127.0	130.7	—	—	—	—	—	127.0	—	126.4
5‴	141.4	138.7	142.5	—	—	—	—	—	138.4	—	141.9
6‴ a)	30.0	125.3	130.4	—	—	—	—	—	125.4	—	29.1 a)
7‴ a)	29.0	141.0	143.8	—	—	—	—	—	140.7	—	28.4 a)
8‴	32.4	74.1	74.0	—	—	—	—	—	74.1	—	31.4
9‴	23.4	31.0	31.1	—	—	—	—	—	31.0	—	22.5
10‴	14.4	10.2	10.1	—	—	—	—	—	10.1	—	14.0

a) No exact assignment possible

Scheme 3. Degradation of the spirocyclic diglycoside **7**.



Scheme 4. Selective base catalysed hydrolysis of papulacandin B (**1**).



ring is in an axial position.

For establishing the connection of the spirocyclic diglycoside **7** with the two fatty acids **5** and **6**, the interpretation of the 360-MHz-^{1}H-NMR-, ^{13}C-NMR- and the EI mass spectra of papulacandin B (**1**), its tetradecahydro derivative and several of the already mentioned degradation products of **1** gave the required information. The ^{1}H-NMR results are briefly summarized in Table 1: by comparison of the chemical shifts found in the diglycoside nonaacetate **7a** with the known ones of β-O-methyl-6-galactopyranoside[7], the protons at C (1′) to C (6′) could be identified. On the other hand, with the aid of decoupling experiments, the protons at C (2) to C (6) could be located in the spectra of the degradation product **10** and its tetraacetate **10a**. Thus, by means of this comparison all the protons in the spirocyclic diglycoside **7** could be firmly assigned.

In a subsequent step the corresponding assignment, as before, can then be made in the 360-MHz-^{1}H-NMR-spectrum of tetradecahydropapulacandin B nonaacetate (Table 1). Comparing the chemical shifts of the C (2)- with the C (3)-proton in tetradecahydropapulacandin B and its nonaacetate, the C (3)-protons were both found at low field positions (5.36 and 5.5 ppm) as triplets. About the same chemical shifts were observed for this proton in the sugar nonaacetate **7a** and the tetraacetate **10a**, whereas in the non-acetylated diglycoside **10** this signal appears at much higher field (3.69 ppm). Consequently we must assume that the C (3)-hydroxyl group is acylated in papulacandin B with one of the two fatty acids **5** or **6**. The C (2)-proton was located at 4.33 ppm as a doublet in tetradecahydropapulacandin B, and a similar chemical shift was found for this proton in the degradation product **10** with a free C (2)-hydroxyl group. Therefore, the C (2)-hydroxyl group must be free in papulacandin B.

Because we had also some evidence for an acylation of one of the two primary hydroxyl groups (C (6) or C (6′)) with one of the two fatty acids (**5** or **6**) from the ^{13}C-NMR-spectrum (different chemical shifts for C (6) and C (6′) at 61.6 and 64.9 ppm, respectively; *cf.* Table 2) and also from the 360-MHz-^{1}H-NMR-spectrum of **1** (two multiplets at 4.11 and 4.25 ppm, indicating presence of an acylated primary

hydroxyl group*), a selective base catalysed hydrolysis of **1** was attempted. Beside the already known fatty acid **5** two further products could be isolated, namely compound **12** with the longer fatty acid occupying the C (3) position, and compound **13** in which, due to an acyl migration, the fatty acid is now attached to C (2) (Scheme 4). The following facts are evident from the 360-MHz-^{1}H-NMR spectra of **12** and **13** (Table 1):

In the spectra of both compounds the signals of the shorter fatty acid **5** are absent in contrast to those of the longer one **6**.

The chemical shifts of the C (2)- and C (3)-protons in compound **12** are very similar to our earlier findings in papulacandin B proving that the C (3)-hydroxyl group is esterified with the longer fatty acid **6**.

These results are supported by the fact that in the NMR-spectrum of the rearranged product **13** the signal of the C (3)-proton is missing at low field, *i.e.* it is shifted to higher field (4.07 ppm) due to the now free hydroxyl group. The C (2)-proton signal, on the other hand, is shifted downfield from 4.33 to 5.68 ppm due to acylation.

In both the hydrolysis products **12** and **13** the two multiplets as 4.11 and 4.25 ppm of the acylated primary hydroxyl group and the triplet of the adjacent single proton at 3.66 ppm were altogether shifted to higher field (not located there) due to the formation of a free hydroxyl function. This is compelling evidence for an attachment of the shorter fatty acid **5** to one of the two primary hydroxyl groups (C (6) or C (6′)) of the overall sugar portion of the molecule.

In the ^{13}C-NMR-spectra of both **12** and **13** the chemical shifts for the C (6) and C (6′) carbons are very similar (**12**: 61.6 and 62.0 ppm; **13**:

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

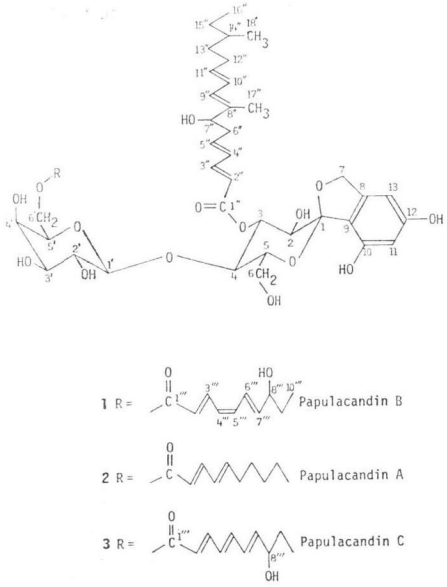
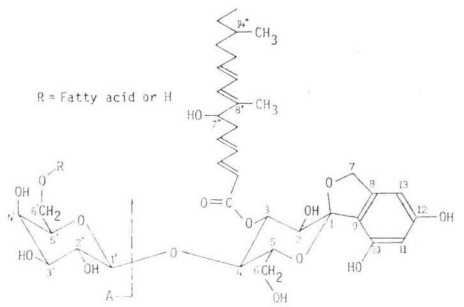


Fig. 2. Characteristic mass spectral fragmentation of papulacandin derivatives.



Compound	Fragment A (*m/z*)*	
Papulacandin B nonaacetate	A(ac)$_4$	495
Papulacandin B nonaacetate (d$_3$)	A(ac-d$_3$)$_4$	507
Papulacandin B dimethyl ether heptaacetate	A(ac)$_4$	495
12a (nonaacetate) (R=H)	A(ac)$_4$	331
Papulacandin B, (TMS)$_9$-derivative	A(TMS)$_4$	615
Tetradecahydropapulacandin B nonaacetate	A(ac)$_4$	501
Papulacandin A octaacetate	A (ac)$_3$	439
Papulacandin A, (TMS)$_8$-derivative	A(TMS)$_3$	529

* TMS=trimethylsilyl, ac=acetate

* The coupling with an adjacent single proton (triplet at 3.66 ppm) was demonstrated by a decoupling experiment.

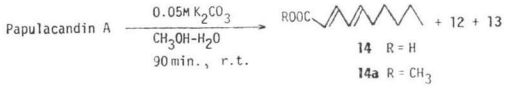
61.4 and 62.7 ppm, compared to 61.6 and 64.9 ppm in **1**).

A clear-cut decision as to which of the two potential acylation sites, C (6) or C (6′), carries the shorter fatty acid **5** could be made on the basis of the EI (electron impact) mass spectra of several derivatives* of **1**. As one would expect, a major fragmentation process involves simple α-cleavage of the glycosidic bond at the galactose moiety, severing the two sugar units in the desired fashion (Fig. 2). By this process fragments **A**, *i.e.* galactosyl oxonium ions, are generated which, invariably, contain the shorter acid **5** except when removed by partial hydrolysis such as in **12a**. Thus, in the nonaacetate of papulacandin B an **A** fragment is observed at *m/z* 495 due to acetylation of three free sugar hydroxyl groups in addition to a fourth one present at C (8‴) of the fatty acid chain; this fragment is shifted by 12 units up to *m/z* 507 on deutero-acetylation, to *m/z* 501 after hydrogenation of **1** (tetradecahydro derivative) due to an uptake of 3 mole H_2 by the triply unsaturated acyl residue, and down to *m/z* 331 after partial hydrolysis (**12a**), reflecting the replacement of the acyl substituent by the much lighter acetyl group. Trimethylsilylation as an alternative to acetylation in preparing sufficiently volatile derivatives gives analogous results. By dismissing C (6) in favor of C (6′) as the actual acylation site, these findings establish **1** as the structure of papulacandin B.

Structure of Papulacandin A (2)

Papulacandin A ($C_{47}H_{66}O_{16}$) differs from papulacandin B only in the structure of the shorter fatty acid by lacking the third conjugated double bond in addition to the hydroxyl group. Elucidation of the structure of **2** was achieved in the same way as previously described for papulacandin B. Selective alkaline hydrolysis (Scheme 5) gave, in addition to 2, 4-decadienoic acid (**14**), again the two products **12** and **13** lacking the fatty acid **14** at the primary hydroxyl group of the galactose unit. The structure of the acid **14** was determined by analysis of the 360-MHz-^{1}H-NMR, ^{13}C-NMR and mass spectra of its methyl ester **14a** ($C_{11}H_{18}O_2$, $M^{+\cdot}$ at *m/z* 182). As opposed to **5** and **6**, this acid has already been described in the literature, *i.e.* as a fragment of the antileukemic diterpenoid gnididin[8].

Scheme 5. Selective base catalysed hydrolysis of papulacandin A (**2**).



The same conclusions as for papulacandin B can be made with respect to the positions of attachment of the two fatty acids:

In the 360-MHz-^{1}H-NMR spectra of papulacandin A (**2**) and its dodecahydro derivative the C (3)-proton was found as a low field triplet at 5.38 ppm and 5.32 ppm, respectively, thus demonstrating also for **2** that the C (3)-hydroxyl group is acylated (Table 1).

The C (2)-protons, on the other hand, were identified in papulacandin A and its perhydro derivative as doublets at 4.33 and 4.30 ppm, respectively, thus confirming a free C (2)-hydroxyl group.

The C (5′)- and C (6′)-protons were found at very similar chemical shifts as in papulacandin B: the C(5′)-proton as a triplet at 3.66 ppm and the C (6′)-proton as two multiplets at 4.13 and 4.21 ppm, thus showing that, in analogy to **1**, the primary hydroxyl group of the galactose moiety is acylated.

As shown in Fig. 2, the EI mass spectra of papulacandin A octaacetate ($C_{63}H_{82}O_{24}$, highest mass

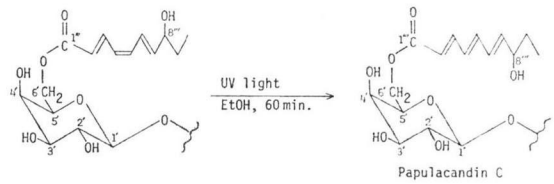
* Not surprisingly, no EI spectra could be obtained on papulacandins as such due to thermal decomposition. Analysis of underivatized papulacandins by FD (field desorption) mass spectrometry gave correct molecular weight information and characteristic fragments reflecting the galactosyl residue as well as the remaining portion of the molecule. Poor reproducibility did, however, not warrant to rely on them too heavily or exclusively.

peak m/z 1162, $M^{+\cdot}-AcOH$) and the corresponding $(TMS)_8$ derivative ($C_{71}H_{131}O_{16}Si_8$, $M^{+\cdot}$ at m/z 1462) exhibit characteristic A fragments at m/z 439 and 529, respectively. These m/z values of the galactosyl ions clearly indicate the substitution by a fatty acid (**14**) now being 14 u lighter than **5** in **1**, which amounts to a loss of the hydroxyl group along with a gain of 2 hydrogens. This lack of the hydroxyl group is, of necessity, also reflected in the presence of only 3 acetate or silyl groups introduced on derivatization, as opposed to the introduction of **4** such groups in the case of papulacandin B.

With these data structure **2** for papulacandin A is well established.

Structure of Papulacandin C (3)

As one can see from Table 2 the ^{13}C-NMR-data of papulacandin C are very similar to those of papulacandin B. However, papulacandin B can easily be transformed into papulacandin C by exposing an alcoholic solution of papulacandin B to UV-light. Therefore we believe that in **3** the double bonds of the shorter fatty acid are in the all-*trans* configuration as opposed to *trans-cis-trans* in papulacandin B.

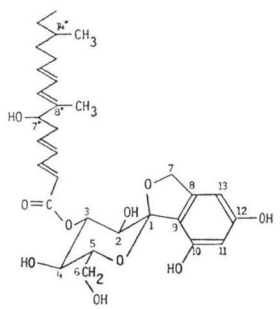


It is very likely that papulacandin C is not a genuine product of the fermentation of *Papularia sphaerosperma* but an artifact formed during the isolation procedure.

Structure of Papulacandin D (4)

In papulacandin D (**4**, $C_{31}H_{42}O_{10}$, Fig. 3) the galactose moiety of the three previous papulacandins together with their respective fatty acids is absent. The elemental composition is in full accord with the EI mass spectrum of a $(TMS)_6$ derivative ($C_{49}H_{90}O_{10}Si_6$, $M^{+\cdot}$ at m/z 1006). The structure of papulacandin D can directly be deduced from the ^{13}C-NMR and 360-MHz-^1H-NMR spectra (Tables 1 and 2) by comparison with the corresponding spectra of papulacandin B (**1**).

Fig. 3. Structure of papulacandin D.



4

In the ^{13}C-NMR spectrum (Table 2) of **4** all signals of the galactose as well as those of the shorter fatty acid are missing, yet the chemical shifts of the remaining C-atoms are very close to those found for **1**.

In the 360-MHz-^1H-NMR spectrum the C (3)-proton is, again, found at low field (5.34 ppm) due to acylation of the 3-hydroxyl group; by contrast, the C (2)-proton is found at much higher field (4.34 ppm) due to the presence of a free hydroxyl group at this site.

These findings lead compellingly to structure **4** for this congener.

Discussion

The structures of the papulacandins are obviously highly amphophilic. The hydrophilic portion

of the molecule contains glucose and, in all but one case, galactose residues, each carrying one strongly lipophilic chain of a higher unsaturated fatty acid as an acyl substituent at a secondary and primary hydroxyl group, respectively. Several well known classes of antibiotics bear structural resemblances with the papulacandins as far as building principle and building blocks are concerned. *E.g.*, polyene antibiotics frequently contain highly unsaturated macrocyclic ring portions (in addition to highly hydroxylated ones) in conjunction with sugar and amino residues[9]. Tunicamycin contains two substituted glucosamine moieties and a long-chain unsaturated fatty acid[10]; moenomycin and a number of related antibiotics contain even three sugar moieties in addition to a long-chain unsaturated phosphoric ester[11].

Despite these structural similarities with other antibiotics, the intimate connection of a sugar moiety with an aromatic nucleus *via* C-O and C-C bonds forming a spirocyclic interface represents, nonetheless, a structural element of novelty within the antibiotics literature, justifying to view the papulacandins as a new class. Their good *in vitro* activity, their mode of action as inhibitors of glucan synthesis during cell wall synthesis in yeast[3], and - last not least - their low toxicity warrant the pursuit of further biological and chemical investigation.

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 the 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 with regard to TMS as internal standard. Abbreviations: s=singlet, d=doublet, dd=double doublet, t=triplet, q=quartet, m=multiplet. FD (field desorption) and EI (electron impact) mass spectra were recorded with a Varian MAT CH 5 DF mass spectrometer equipped with a FD/FI/EI combination source.

For column chromatography silica gel Merck 60 (0.063 ~ 0.20 mm) or Sephadex LH-20 (Pharmacia) was used. For thin-layer chromatography silica-gel plates F_{254} (Merck) were used.

Base catalysed hydrolysis of papulacandin B (**1**)

A solution of 1,650 mg **1** in 100 ml CH_3OH and 100 ml 1 N NaOH was stirred at 23°C for 3 hours. The solution was extracted 5 times with ethyl acetate at pH 7.5. The combined extracts were washed with water, dried over Na_2SO_4 and evaporated. The residue (505 mg) was treated directly with CH_2N_2/ether at 0°C to yield crude 7-hydroxy-8,14-dimethyl-2,4,8,10-hexadecatetraenoic acid methyl ester (**6a**). Preparative thin-layer chromatography with $CHCl_3$ - CH_3OH (98: 2) gave 120 mg **6a** as a pale yellow unstable oil. UV: λ_{max}^{EtOH} nm(ε): 240 (27,400), 259 (25,100). IR: $\nu^{CH_2Cl_2}$: 1710, 1645, 1615, 1460, 1430 cm^{-1}. MS: *m/z* 306 ($M^{+\cdot}$, $C_{19}H_{30}O_3$). 360-MHz-NMR ($CDCl_3$, δ in ppm): H (2′): 5.80 d (J=16); H (3′): 7.25 dd (J=12/16); H (4′): ~6.2 m; H (5′): 6.10 dd (J=8/16); H (6′): 2.42 t (J=7); H (7′): 4.12 d (J=7); H (9′): 5.98 d (J=12); H (10′): ~6.2 m; H (11′): 5.68 m (J=8/16); H (12′): ~2.1 m; CH_3 (17′): 1.75 s. ^{13}C-NMR ($CDCl_3$): Table 2.

The aqueous solution remaining after ethyl acetate extraction was acidified to pH 2.5 and extracted several times, again, with ethyl acetate. The combined extracts were washed with water, dried over Na_2SO_4 and evaporated. The residue (525 mg) was treated directly with CH_2N_2/ether at 0°C to yield crude **5a**. Preparative thin-layer chromatography, as above, gave 75 mg **5a** as a pale yellow unstable oil. UV: λ_{max}^{EtOH} nm (ε): 299 (12,000).

IR: $\nu_{max}^{CH_2Cl_2}$: 3550, 2970, 1710, 1620 cm^{-1}. 360-MHz-NMR ($CDCl_3$, δ in ppm): H (2‴): 5.90 d (J=14); H (3‴): 7.80 dd (J=11/14); H (4‴): 6.11 t (J=11, *cis*); H (5‴): 6.33 t (J=11, *cis*); H (6‴): 6.83 dd (J=11/15); H (7‴): 5.92 dd (J=6/15); H (8‴): 4.20 q (J=6). ^{13}C-NMR ($CDCl_3$): Table 2. MS: *m/z* 196 ($M^{+\cdot}$, $C_{11}H_{16}O_3$).

To isolate the diglycoside **7** the aqueous solution was neutralized with 1 N NaOH and evaporated to dryness. The residue was chromatographed twice on Sephadex LH-20 (150 g) with methanol as eluent. After precipitation from methanol-ether 250 mg **7** was obtained as colourless water-soluble material. $[\alpha]_D^{23}$ 31±1° (*c* 0.25, CH_3OH). UV: λ_{max}^{EtOH} nm (ε): 222 (7200), 273 (1300), 278 (1300). ^{13}C-NMR

(CD_3OD): Table 2.

Acetylation of **7** with acetic anhydride-pyridine gave the nonaacetate **7a** (m.p. 119~123°C). UV: λ_{max}^{EtOH} nm (ε): 261 (420). IR: ν_{max}^{KBr}: 1755, 1375, 1220, 1125 cm^{-1}. ^1H-NMR ($CDCl_3$) (Table 1). ^{13}C-NMR ($CDCl_3$) (Table 2). MS: *m/z* 840 ($M^{+\cdot}$, $C_{37}H_{44}O_{22}$), *m/z* 781 ($M^{+\cdot}-OAc$).

Calcd. for $C_{37}H_{44}O_{22}$: C 52.86, H 5.24, O 41.90%.
Found: C 53.05, H 5.63, O 41.80%.

Ozonolysis of **6a**

65 mg **6a** in CH_2Cl_2 was treated with ozone at −70°C until the solution had turned blue. The solution was evaporated to dryness, 5.5 ml CH_3COOH, 1.2 ml 30% H_2O_2 and 0.1 ml conc. HCl were added, and the solution kept for 20 hours at 23°C. After evaporation to dryness the residue was dissolved in ether and treated with CH_2N_2/ether at 0°C for 15 minutes. After filtration and evaporation the residue was dissolved in 0.25 ml ether. Dimethyl oxalate and methyl 4-methyl-caproate could be identified through gas chromatographic analysis by comparison with authentic samples.

Degradation of the diglycoside **7**

Methylation: 1 g **7** in 100 ml DMF was stirred vigorously with 5 g Ag_2O and 3.1 g CH_3J at 23°C for 90 minutes. The solution was filtered over Celite and evaporated to dryness. The residue was chromatographed on silica-gel (150 g) with $CHCl_3$ and increasing proportions of CH_3OH (5~50%) as eluents. 600 mg dimethyl ether **8** was obtained. UV: λ_{max}^{EtOH} nm (ε): 271 (125), 277 (130). IR: ν_{max}^{KBr}: 3500, 2950, 1610, 1495, 1470, 1434 cm^{-1}. ^1H-NMR (CD_3OD, δ in ppm): H (11) and H (13): 6.5 s; 2× O-CH_3: 3.85 and 3.87. MS (heptaacetate, $C_{35}H_{44}O_{20}$): *m/z* 784 ($M^{+\cdot}$); *m/z* 725 ($M^{+\cdot}-OAc$).

Methanolysis: 120 mg **8** in 10 ml 1.5 N methanolic HCl solution was allowed to react for 63 hours at 23°C. The solution was carefully evaporated to dryness. Preparative thin-layer chromatography of the residue ($CHCl_3$ - CH_3OH, 2: 1) and elution of the faster running zone with methanol gave 39 mg of the glycoside **10** as a colourless oil. IR: $\nu_{max}^{CHCl_3}$: 3400, 2950, 1605, 1500, 1470, 1430, 1350, 1160, 1090, 1070, 1005 cm^{-1}. 360-MHz-NMR (CD_3OD): Table 1, ^{13}C-NMR (CD_3OD): Table 2. MS: *m/z* 328 ($M^{+\cdot}$, $C_{15}H_{20}O_8$).

Acetylation of **10** in acetic anhydride-pyridine gave the tetraacetate **10a**. MS: *m/z* 496 ($M^{+\cdot}$, $C_{23}H_{28}O_{12}$).

Elution of the slower running zone (Rf 0.05) with methanol, and acetylation with acetic anhydride-pyridine gave a mixture of methyl-α- and methyl-β-D-galactopyranoside tetraacetate which was separated by preparative thin-layer chromatography on silica-gel with ether - hexane (2: 1). After crystallization from ether - hexane 21 mg methyl-α-D-galactopyranoside tetraacetate was obtained, m.p. 122°C. $[\alpha]_D$, IR, MS and NMR were identical with corresponding data of a reference sample.

Periodic acid cleavage: A solution of 9 mg **10** and 34 mg H_5JO_6 (5 equiv.) in 1 ml H_2O was allowed to react at 23°C for 16 hours. After filtration the aqueous solution was extracted five times with ether. The combined ether extracts were dried and evaporated. The residue (3 mg) showed only one spot in TLC ($CHCl_3$ - CH_3OH, 97: 3). The resulting 5,7-dimethoxyphthalide (**11**) had the following spectroscopic properties: IR: $\nu_{max}^{CH_2Cl_2}$: 2980, 1740, 1600, 1450, 1345, 1160, 1090, 1050, 1040, 1015 cm^{-1}. ^1H-NMR ($CDCl_3$, δ in ppm): 6.5 s (1 H, arom.), 6.48 s (1 H, arom.), 5.2 s (H-7), 3.92 s and 3.98 s (2×O-CH_3). MS: *m/z* 194 ($M^{+\cdot}$), 176 ($M^{+\cdot}-H_2O$), 165 ($M^{+\cdot}-CHO$).

Selective base catalysed hydrolysis of papulacandin B (**1**) and A (**2**)

A solution of 4 g papulacandin B (**1**) in 200 ml 0.05 M K_2CO_3 in CH_3OH - H_2O (1: 1) was stirred at 23°C for 3 hours. The aqueous solution was extracted five times with ethyl acetate at pH 7.5. The combined ethyl acetate extracts were washed, dried over Na_2SO_4 and evaporated to yield 3.1 g of an oily residue. Chromatography of the residue on 150 g silica-gel with $CHCl_3$ and increasing proportions of CH_3OH gave the free acids **5** and **6** together with their methyl esters **5a** and **6a** in the lipophilic fractions, and 1,850 mg of a mixture of the selective degradation products **12** and **13** in the more polar fractions ($CHCl_3$+50% CH_3OH). After preparative TLC of the latter mixture on 10 silica-gel plates (20×100 cm) with $CHCl_3$ - CH_3OH (7: 3) and precipitation from ether - hexane, 700 mg **12** and 500 mg of the rearranged product **13** were obtained as colourless materials.

Spectroscopic properties of **12** and **13**

12 ($C_{37}H_{52}O_{15}$): UV: ν_{max}^{EtOH} (ε): 232 (33,800), 238 (shoulder), 260 (22,200). IR: ν_{max}^{KBr}: 3500, 2970, 1705, 1695, 1615, 1465, 1385, 1353, 1265, 1155 cm^{-1}. 360 MHz-NMR and ^{13}C-NMR (CD_3OD): Tables 1 and 2. MS (nonaacetate $C_{55}H_{70}O_{24}$): *m/z* 1,054 ($M^{+\cdot}$ −HOAc).

13($C_{37}H_{52}O_{15}$): UV: λ_{max}^{EtOH} (ε): 230 (27,600), 238 (27,600), 250 (27,200). IR: ν_{max}^{KBr}: 3500, 2970, 1705, 1690, 1660, 1385, 1270, 1153 cm^{-1}. 360-MHz-NMR and ^{13}C-NMR (CD_3OD): Tables 1 and 2. MS (nonaacetate $C_{55}H_{70}O_{24}$): *m/z* 1,054 ($M^{+\cdot}$ −HOAc).

From an analogues selective basic hydrolysis of 5 g papulacandin A (**2**) 200 mg methyl ester **14a**, 900 mg **12** and 600 mg **13** were obtained.

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