

THE STRUCTURE OF VACIDIN A, AN AROMATIC HEPTAENE MACROLIDE ANTIBIOTIC

I. COMPLETE ASSIGNMENT OF THE ^1H NMR SPECTRUM AND GEOMETRY OF THE POLYENE CHROMOPHORE

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The constitution of vacidin A, a representative of the aromatic heptaene macrolide antibiotics was established on the basis of ^{13}C and ^1H - ^1H double quantum filtered correlated spectroscopy, rotating frame nuclear Overhauser effect spectroscopy, J -resolved ^1H as well as ^1H - ^{13}C correlation NMR spectra. Geometry of the polyene chromophore was determined as $22E,24E,26E,28Z,30Z,32E,34E$.

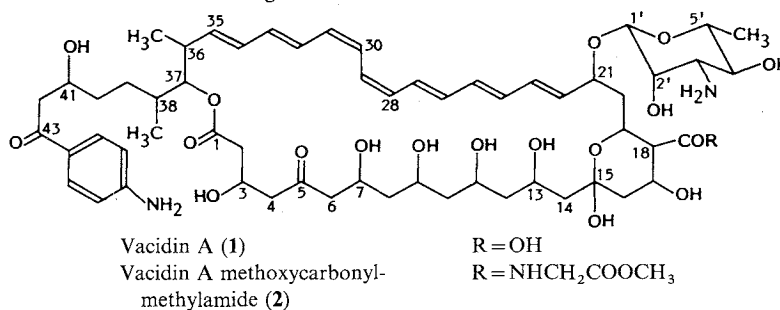
Vacidin A and gedamycin¹⁾ are the main components of the aureofacin complex produced by *Streptomyces aureofaciens*²⁾. These two antibiotics belong to the aromatic heptaene macrolide group. Gross structures of vacidin A and gedamycin, elucidated by MS, were proposed by ZIMIŃSKI *et al.*¹⁾. Subsequently, it was shown that structures of partricins A and B were identical with the constitution of vacidin A and gedamycin^{3,4)}.

Our recent NMR studies of vacidin A confirmed the earlier structural assignment for this antibiotic and allowed us to determine its absolute configuration⁵⁾.

For our work we used the methoxycarbonylmethylamide derivative of vacidin A (VacGlyOMe, **2**)⁶⁾, Fig. 1. It follows from our investigations that, this type of derivative is recommendable for NMR studies due to improved physico-chemical properties such as increased solubility in polar solvents along with satisfactory stability in solution. Moreover, higher lipophilicity of this type of derivative allowed simplification of the isolation by replacement of the very laborious CCD method with silica gel column chromatography.

Taking proton signal differentiation as a criterion, several NMR solvents were tested and the solvent

Fig. 1. Structure of vacidin A.



system pyridine- d_5 with 10% methanol- d_4 was selected for further studies. The natural line width was 1.8 Hz, which was very satisfactory for this type of compound.

Results and Discussion

The ^{13}C NMR spectrum of **2** showed sixty two carbon signals of which partial assignments, shown in Table 1, were made based upon distortionless enhancement by polarization transfer (DEPT), ^1H - ^{13}C correlation experiments and literature data describing ^{13}C NMR analysis of polyene macrolides⁷.

The data from ^1H NMR studies of **2**, which included double quantum filtered phase sensitive correlated spectroscopy (DQF-COSY)^{8,9} and rotating frame nuclear Overhauser effect spectroscopy^{10,11} (ROESY) experiments (Fig. 2) are collected in Table 2. The latter, in contrast to nuclear Overhauser enhancement and exchange spectroscopy (NOESY), always feature positive NOE's (negative cross-peaks with respect to diagonal), eliminating known problems of NOE's vanishing or spin diffusion, depending on correlation time, when high field spectrometers are used for measurements of medium size compounds. Wide-spread spin diffusion was observed for another compounds of this group, amphotericin B¹²) (1D NOE experiment in DMSO, 300 MHz).

The coupling constants listed in the Table 2 were assigned on the basis of 1D ^1H NMR spectrum of **2**, but for a few cases the analysis of phase structure of the cross-peaks in the DQF-COSY spectrum was carried out to attribute correct values to the appropriate protons. Also, the analysis of the NOE effects yields the same results.

Analysis of a DQF-COSY spectrum of **2** allowed us to distinguish in **2** five structural blocks as C-2–C-4, C-6–C-14, C-16–C-42, aromatic and sugar moieties, which were separated by non protonated carbons (C-1, C-5, C-15, C-43) and glycosidic linkage. Within these blocks all but one cross peaks have been observed. Although a cross peak 20- H_a –19-H was not found in DQF-COSY this connectivity was fully confirmed in a ROESY spectrum.

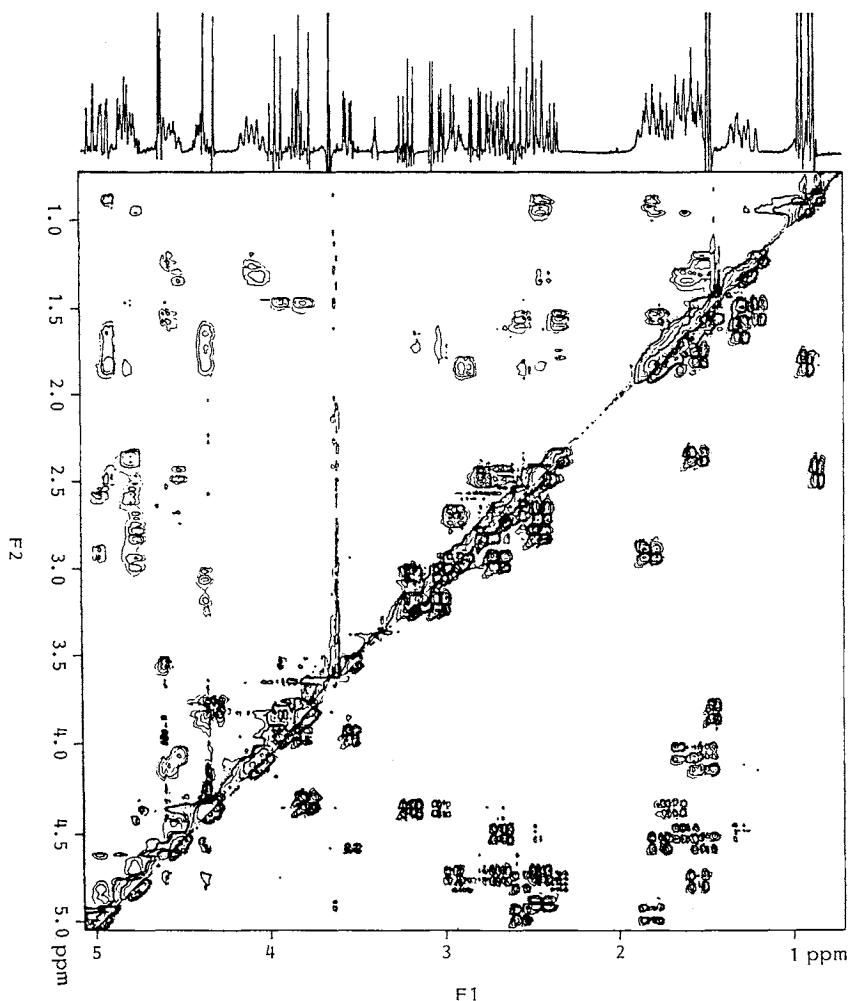
It was found to be a general pattern that for weak cross-peak signals in DQF-COSY, strong NOE's

Table 1. ^{13}C NMR data for vacidin A methoxycarbonylmethylamide (**2**).

Description	No. of carbon atoms	δ (ppm)	Description	No. of carbon atoms	δ (ppm)
CH_3	4	13.5, 16.7, 18.6, 52.4	27		128.58
CH_2	13	31~52	28		130.91
$>\text{CH}-$			29		125.53
CHCH_3	2	34.3, 40.4	30 (24)		125.28
CHNH_2	1	57.9	31		130.84
CHCONHR	1	59.3	32		128.62
CHOR	13	64~79	33 (23)		134.42
Acetal	1	97.7	34		133.67
CH Ar	4	113.7, 131.6	35		137.88
$=\text{CH}-$ (olefinic) ^a :			Non protonated:		
22		137.67	Hemiketal	1	98.2
23 (33)		130.00	C Ar	2	127.0, 171.2
24 (30)		134.80	COXR	3	171.8, 174.6, 174.7
25		133.10	C=O	2	198.1, 208.9
26		135.64			

^a Assignment by H,C-COSY. Interchangeable assignments are shown in parentheses.

Fig. 2. Spectra of vacidin A methoxycarbonylmethylamide.



Spectral region 0.72~5.06 ppm of 300 MHz ROESY (upper triangle) and DQF-COSY (lower triangle) spectra of VacGlyOMe (15 mg/ml, pyridine- d_5 -methanol- d_4 , 9:1) combined along the diagonal.

in a ROESY spectrum were observed (Table 2). Such effects can be expected in the case when dihedral angles between vicinal protons are in the range $60\sim 90^\circ$.

Thus, full assignment has been made for five structural blocks of **2** which were assembled in the next step of our studies where NOE's observed in ROESY spectra of **2** were considered.

Hence, the mutual orientation of C-6-C-14 and C-16-C-42 fragments resulted from NOE between 14-Ha and 16-Ha. 38-CH₃-3-H and 3-H-34-H NOE's combined with the chemical shift of 37-H (4.94 ppm) characteristic of an acyloxy proton pointed out that C-2-C-4 and C-16-C-42 fragments were separated by an ester moiety located between C-2 and C-37. The connections of mycosamine and aromatic moieties with the C-16-C-42 fragment were indicated by 1'-H-21-H and 42-H-aromatic proton NOE's, respectively. Although 4-H-6-H NOE's were not observed in the ROESY spectrum of **2** the connection of C-2-C-4 and C-6-C-14 fragments was deduced as follows. The chemical shifts of the protons at C-4 and C-6 methylene groups indicated that they were located next to a carbonyl group. There were five carbonyl

Table 2. ¹H NMR data for the vacidin A, NOE effects^a.

No. of proton	δ (ppm)	$J_{H,H}$ (Hz) (coupling partner)	NOE to protons (intensity)
2a	2.80	15.1 (2b), 3.7 (3)	3 (m)
2b	2.45	15.1 (2a), 9.3 (3)	3 (w)
3	4.78	9.3 (2b), 8.1 (4b), 5.6 (4a), 3.7 (2a)	38-CH ₃ (w), 2a (m), 4a (m), 4b (m), 2b (w), 34 (m)
4a	2.96	17.5 (4b), 5.6 (3)	3 (m), 4b (s)
4b	2.68	17.5 (4a), 8.1 (3)	3 (m), 4a (s)
6a	2.68	16.8 (6b), 9.6 (7)	6b (s), 8a (w)
6b	2.44	16.8 (6a), ~2 (7)	6a (s), 8b (w), 7 (m)
7	4.54	9.6 (6a), 9.6 (8a), ~2 (6b), ~2 (8b)	8b (m), 6b (m), 9 (s), 28 (m), 29 (m)
8a	1.62	~13 (8b), 9.6 (7), 10.0 (9)	6a (w)
8b	1.30	~13 (8a), ~2 (7), ~2 (9)	7 (m), ^c (m), 6b (w)
9	4.06	10.0 (8a), 10.0 (10a), ~2 (8b), ~2 (10b)	[10b or 8b] ^c (m), 7 (s), 28 (m)
10a	1.51	~13 (10b), 10.0 (9), 10.3 (11)	
10b	1.27	~13 (10a), ~2 (9), ~2 (11)	^c (m), ^d (m)
11	4.11	10.3 (10a), 10.3 (12a), ~2 (10b), ~2 (12b)	[12b or 10b] ^d (m), 13 (s), 26 (m)
12a	1.51	10.3(11), 10.5 (13), 13.5 (12b)	
12b	1.21	~2 (11), ~2 (13), 13.5 (12a)	13 (m), ^d (m)
13	4.59	10.5 (12a), 10.5 (14a) ^b , 2 (12b), 2 (14b) ^b	12b (m), 14b (m), 11 (s), 23 (w), 22 (m), 24 (m)
14a	1.78	10.5 (13)	16a (w)
14b	1.54	2 (13)	13 (m)
16a	2.35	12.2 (16b), 4.7 (17)	16b (m), 14a (w), 17 (m)
16b	1.56	12.2 (16a), 10.3 (17)	18 (m), 16a (m)
17	4.82	10.3 (16b), 10.3 (18)	16a (m), 18 (m), 19 (m)
18	2.56	10.3 (17), 10.1 (19)	16b (m), 20b (w), 19 (m), 17 (m)
19	5.00	10.1 (18), 10.1 (20b) ^b	18 (m), 20 (m), 2' (m), 1' (m), 17 (m), 23 (w), 22 (m)
20a	2.90	~9 (21)	20b (m), 21 (w), 19 (m), 1' (m)
20b	1.82	10.1 (19)	18 (w), 21 (w), 20 (m)
21	4.83	9 (22), ~9 (20a) ^b	20a (w), 20b (w), 1' (s), 23 (s), 22 (m)
22	6.36	9 (21), 15 (23)	19 (m), 21 (m), 24 (w), 13 (m)
23	6.24	11 (24), 15 (22)	19 (w), 21 (s), 13 (w), [24 or 30 or none] ^e (w)
24	6.64	11 (25), 15 (23)	13 (m), 22 (w), ^e (w)
25	6.37	11 (26), 15 (24)	27 (m)
26	6.68	11 (25), 15 (27)	11 (m), 28 (m)
27	6.95	11.5 (28), 15 (26)	30 (m), 25 (m)
28	6.51	11.5 (27), 11.5 (29)	7 (m), 9 (m), 26 (m), 29 (s)
29	6.97	11.5 (28), 11.5 (30)	7 (m), 28 (s), 32 (s)
30	6.63	11.5 (29), 11.5 (31)	31 (s), 27 (m), [33 or 23 or none] ^e (w)
31	6.15	11.5 (30), 11.5 (32)	33 (m), 30 (s)
32	7.17	11.5 (31), 15 (33)	34 (m), 33 (w), 29 (s)
33	6.24	11 (34), 15 (32)	35 (m), 31 (m), 32 (w), ^e (w)
34	6.34	11 (33), 15 (35)	35 (m), 3 (m), 36 (s), 32 (m)
35	5.45	9 (36), 15 (34)	36-CH ₃ (m), 37 (m), 33 (m), 34 (m)
36	1.81	9 (35), 9.8 (37)	38-CH ₃ (m), 36-CH ₃ (m), 38 (w), 37 (m), 34 (s)
37	4.94	9.8 (36), 2.2 (38)	36-CH ₃ (m), 38 (m), [40 or 39] ^f (m), 36 (m), 35 (m)
38	1.81	6.7 (39) ^g , 2.2 (37) ^g	36-CH ₃ (m), 38-CH ₃ (m), 36 (w), 37 (m)
39a } 39b }	1.61		^f (m)

Table 2. (Continued)

No. of proton	δ (ppm)	$J_{H,H}$ (Hz) (coupling partner)	NOE to protons (intensity)
40a } 40b }	1.70		^f (m)
41	4.37	8.1 (42a), 4.2 (42b)	40a/b (m), 42a (m), 42b (m)
42a	3.20	15.6 (42b), 8.1 (41)	41 (m), 42b (s)
42b	3.02	15.6 (42a), 4.2 (41)	41 (m), 42a (s)
36-CH ₃	0.87	6.6 (36)	35 (w), 37 (m), 38 (m), 36 (m)
38-CH ₃	0.94	6.8 (38)	3 (w), 38 (m), [39 or 40] ^f (m), 36 (m)
1'	5.14	~0 (2')	20a (m), 3' (s), 5' (s), 2' (s), 21 (s), 19 (m)
2'	4.63	3.3 (3'), 0 (1')	3' (s), 1' (s), 19 (m)
3'	3.55	9.7 (4'), 3.3 (3')	1' (s), 2' (s)
4'	3.97	9.7 (3'), 9.7 (5')	6' (m)
5'	3.82	9.7 (4'), 5.9 (6')	6' (m), 1' (s)
6'	1.45	5.9 (5')	4' (m), 5' (m)
Aromatic protons			
	6.81	8.6	
	7.95	8.6	
Glycine methyl ester protons			
OCH ₃	3.66 (s)		
>CH ₂	3.81/4.35	17.6	

^a NOE scale: 100% for CH₂CO; 10~20% (w), 20~75% (m), 75~200% (s).

^b The correct values of coupling constants were attributed to the appropriate protons by the analysis of antiphase structures of cross-peaks in DQF-COSY spectrum.

^c 10-H_b and 8-H_b have nearly the same chemical shifts. NOE between (10-H_b or/and 8-H_b) and 9-H.

^d 12-H_b and 10-H_b have nearly the same chemical shifts. NOE between (12-H_b or/and 10-H_b) and 11-H.

^e 23-H, 33-H and 24-H, 30-H have in pair nearly the same chemical shifts. NOE between (23-H or 33-H) and (24-H or 30-H).

^f 39-H and 40-H have nearly the same chemical shifts. NOE between (39-H or/and 40-H) and 37-H.

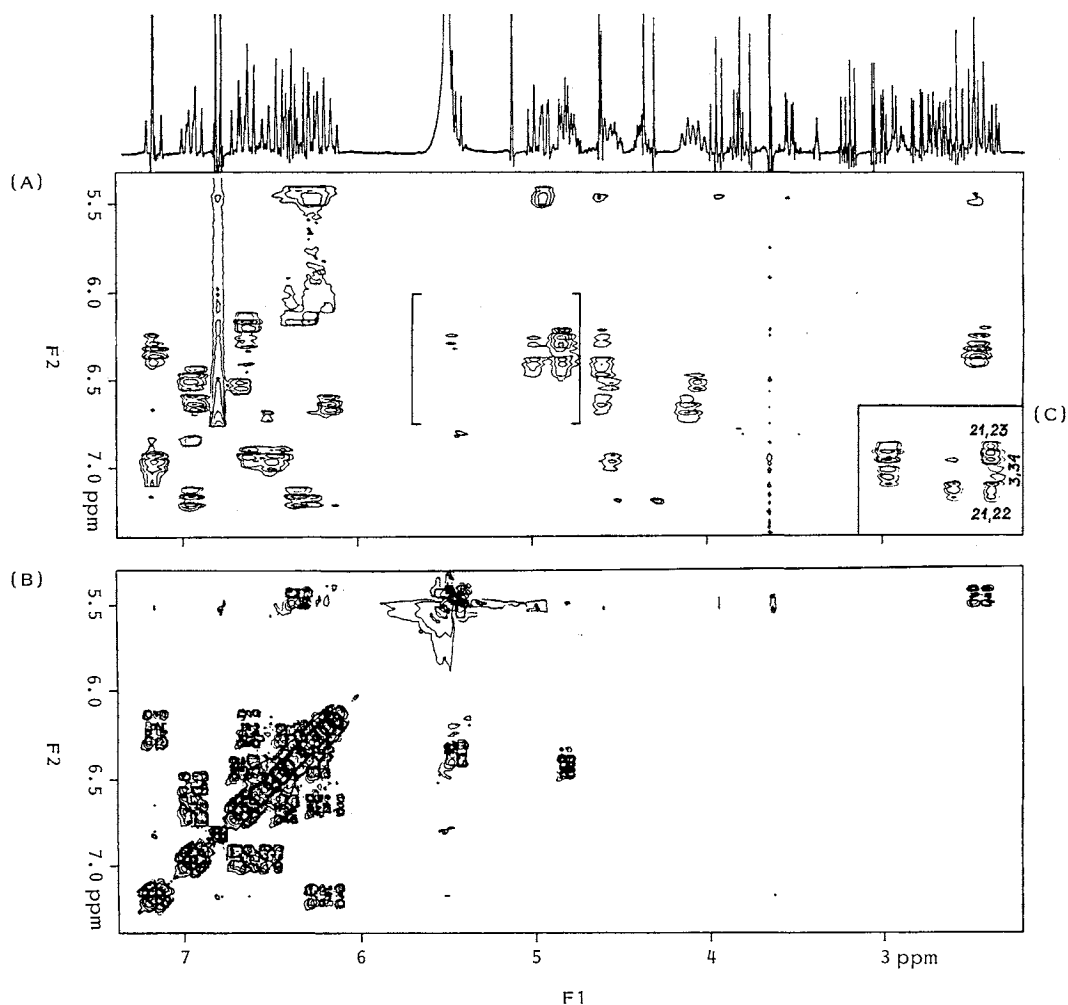
^g Values from ¹H NMR spectrum in 35% DMSO-*d*₆ in methanol-*d*₄.

groups signals observed in the ¹³C NMR spectrum of **2**. Four of them had been already located in the molecule of **2** as a keto group at C-43 and an ester moiety between C-1 and C-37 as well as two carbonyl groups of methoxycarbonylmethylamide moiety at C-18. Thus the remaining carbonyl group must be located at C-5 as a keto group connecting C-2-C-4 and C-6-C-14 fragments.

The locations of the carbonyl groups are additionally supported by geminal coupling constant analysis. The values corresponding to the protons at the carbon atoms adjacent to the carbonyl groups are in the range 15.1~17.5 Hz¹³, in contrast to 12.2~13.5 Hz observed for the rest of methylene groups (Table 2).

The ¹H NMR spectra of nonaromatic heptaenes appeared to be remarkably different from the spectrum of vacidin A in which the signals of olefinic protons occupied almost twice as wide a range of chemical shifts. This prompted us to study the structure of the vacidin A heptaene chromophore. For this purpose ¹H-¹³C correlation and *J*-resolved spectra were used. Although the analysis of olefinic protons in a DQF-COSY spectrum (Fig. 3) alone seemed not to be so promising, four variants of connectivities were found after consideration of the spectral data from ¹H-¹³C correlation and *J*-resolved spectra. Among them, only one set of heptaene proton connectivities justified the NOE effects displayed by the ROESY spectrum of **2** (Fig. 3) and summarized in Fig. 4. The values of vicinal coupling constants measured from *J*-resolved spectrum along with NOE's (ROESY) pointed out the geometry of heptaene chromophore (Fig. 4). The location of two *cis*-double bonds at C-28 and C-30 resulted from the values of $J_{28,29} = 11.5$ Hz

Fig. 3. Spectra showing most of the connectivities of olefinic protons of VacGlyOMe.

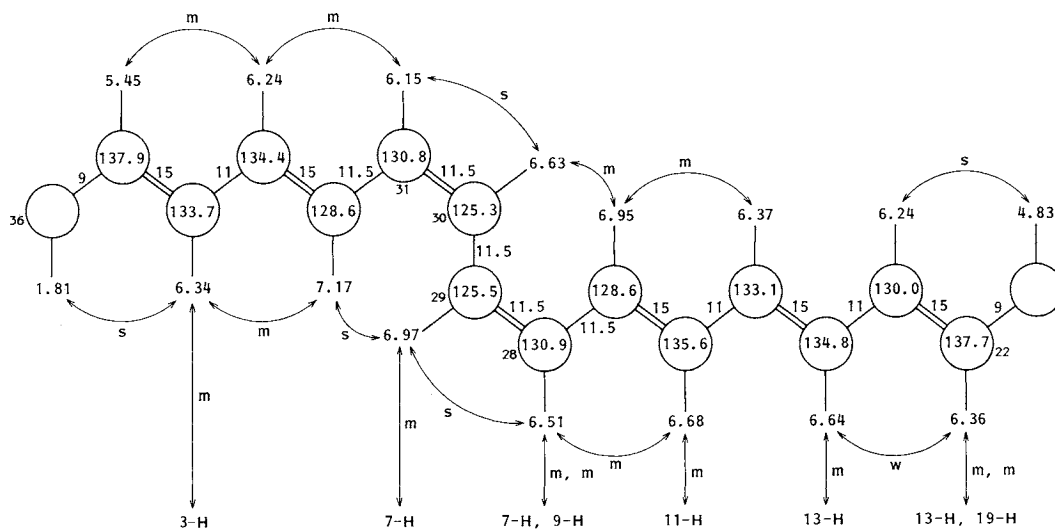


Fragments of A) ROESY and B) DQF-COSY 300-MHz spectra showing most of the connectivities of olefinic protons of VacGlyOMe (15 mg/ml, pyridine- d_5 -methanol- d_4 , 9:1). C) Fragment of ROESY spectrum of VacGlyOMe (90 mg/ml, pyridine- d_5 -methanol- d_4 , 85:15) corresponding to part "A" in brackets. Slightly increased difference between 22-H and 23-H chemical shifts reveals the 3-H–34-H NOE.

and $J_{30,31} = 11.5$ Hz as well as 27-H–30-H, 29-H–32-H, 28-H–29-H and 30-H–31-H NOE effects. Accordingly, the chemical shifts of 27-H, 29-H, 30-H and 32-H protons reflected deshielding effects typical of *cis* double bonds¹⁴. Consequently, C-27, C-29, C-30 and C-32 signals observed in a ^{13}C NMR spectrum of **2** were shielded by approximately 5 ppm due to C-27–C-30 and C-29–C-32 γ effects¹⁵.

It was also observed that the chemical shifts of olefinic protons located inside and outside the macrolactone ring were remarkably different. This could be explained by the interaction of the "internal" protons with the hydrophilic part of the molecule. This effect prevented the occurrence of strongly coupled spin systems and allowed resolution of this part of the spectrum. The above observations permitted us to assign the geometry of the chromophore as 22*E*,24*E*,26*E*,28*Z*,30*Z*,32*E*,34*E*.

Fig. 4. Polyene part of vacidin A.

NOE's, $J_{H,H}$ and 1H , ^{13}C chemical shifts.

Experimental

NMR Spectra

All NMR spectra were recorded with a 300 MHz Varian VXR 300 spectrometer.

Samples were dissolved in pyridine- d_5 -methanol- d_4 (9:1) with a concentration of 15 mg/ml, or 90 mg/ml in pyridine- d_5 -methanol- d_4 (85:15).

The 1H spectrum was measured on the 15 mg/ml sample. The natural line width of OCH_3 signal was 1.8 Hz. The spectrum was recorded with resolution enhancement by Lorentz-Gauss transformation.

^{13}C and DEPT spectra were measured on the 90 mg/ml sample.

(1H - 1H) DQF-COSY spectrum (15 mg/ml) was measured in the phase-sensitive mode. The data were collected in $1,024 \times 256$ matrix and processed in $1,024 \times 1,024$ matrix. The resolution was 4.5 Hz/point.

ROESY spectra (90 mg/ml) were run with a time-shared spin-lock pulse system¹⁶⁾. They were measured under the same conditions with mixing time parameter 0.2, 0.4 and 0.8 second, as well as (15 mg/ml) with mix = 0.4 second.

The J -resolved spectrum (90 mg/ml) was collected in a $2,048 \times 256$ matrix with the spectral window reduced to 844 Hz. The resolution was 0.8 and 0.2 Hz for the δ and J axes, respectively.

The 1H - ^{13}C correlation spectrum (90 mg/ml) was collected in a $1,024 \times 512$ matrix with spectral windows of 1,260 and 450 Hz for ^{13}C and 1H domains, respectively. The resolutions were 2.5 and 1.75 Hz, respectively.

Methoxycarbonylmethylamide of Vacidin A (2)

Methoxycarbonylmethylamide of vacidin A (2) was prepared from crude aureofacin supplied by Pharmaceutical Works Tarchomin-Polfa (Warsaw, Poland), by the procedure previously described⁹⁾. UV λ_{max}^{MeOH} nm ($E_{1\%}^{1cm}$) 378 (1,020).

Acknowledgments

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