VIRIPLANIN A, A NEW ANTHRACYCLINE ANTIBIOTIC OF THE NOGALAMYCIN GROUP

I. ISOLATION, CHARACTERIZATION, DEGRADATION REACTIONS AND BIOLOGICAL PROPERTIES

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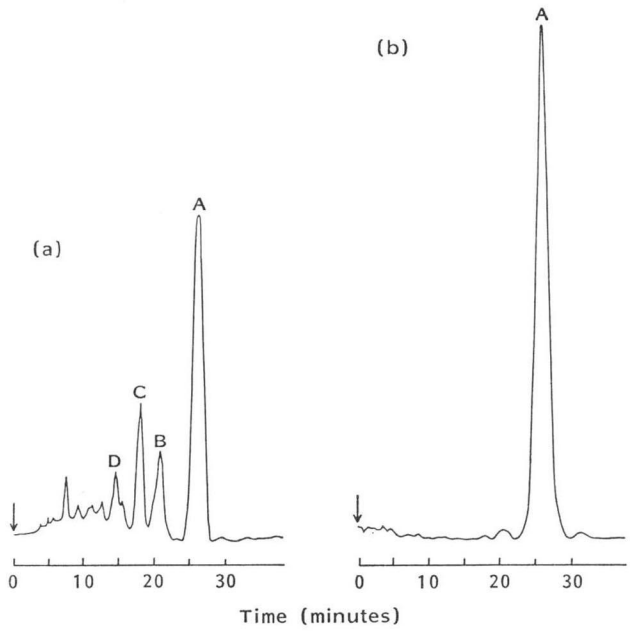
Viriplanin A, a new anthracycline antibiotic produced by *Ampullariella regularis* strain SE 47, was isolated from a raw product that demonstrated activity against Herpes simplex viruses. Based on spectroscopic data, the structure of the aglycone, viriplanol, was determined, and the antibiotic was found to contain the sugar moieties 2-deoxy-L-fucose, 4-*O*-mesaconoyl-L-diginose and decilonitrose. In solution viriplanin A is very unstable to light. The antibiotic belongs to the nogalamycin group and is related to arugomycin and decilorubicin.

During a screening program for antiviral antibiotics of rare actinomycetes we isolated from the culture filtrate of *Ampullariella regularis* SE 47 (ATCC 31417) a mixture of orange-red substances, which showed remarkable activities against Herpes simplex viruses and some Gram-positive bacteria[1]. The primary compound was called viriplanin A. Other components of the mixture seem to be artefacts due to the degradation of viriplanin A during the fermentation and work-up procedure. In this paper we describe the isolation, physico-chemical characterization and biological properties of viriplanin A and give the first structural details of some degradation products.

Isolation

The antibiotic was isolated from the culture filtrate by extraction with butanol at pH 7 and partitioning of the crude material between different solvents. The portion distributing into chloroform - methanol - 33% NH_4OH (120 : 30 : 1) was chromatographed on silica gel with chloroform - methanol - 33% NH_4OH (95 : 5 : 1). The substance from the main zone could be separated into four major components by HPLC analysis on RP-18 silica gel with methanol - 0.01 M phosphate buffer pH 7.2 (65 : 35) as solvent system (Fig. 1a). The main compound (peak A) had the longest retention time (25 minutes) and was called viriplanin A. Its proportion in the raw viriplanin mixture after silica gel chromatography varied from 15 to 30%. The best results were obtained if we excluded light during the

Fig. 1. HPLC analysis of raw viriplanin (a) and of purified viriplanin A (b).



fermentation and isolation procedures. The preparative isolation of viriplanin A became possible by chromatography on RP-8 silica gel columns (Lobar) in the solvent system given above. The purity of the substances was controlled by analytical HPLC (Fig. 1b).

Repeated chromatography under the same conditions could not improve the purity of viriplanin A fraction. It is presumed that viriplanin A begins to degrade during chromatography.

Physico-chemical Characterization of Viriplanin A

Viriplanin A is a red amorphous powder (dec above 210°C), insoluble in diethylether or hexane, soluble in methanol, dimethylsulfoxide, 2 N aqueous NaOH or HCl. In accordance with other anthracycline antibiotics viriplanin A functions as a pH-indicator. Treatment with bases gave a violet color (λ_{max} 539 nm) changing to red-orange (λ_{max} 470 nm) in acidic solutions (Table 1). Thus the chromophore appeared to be very similar to that of nogalamycin (**1a**)[2,3]. In Table 2 the Rf values of viriplanin A in different solvents are compared with those of nogalamycin (**1a**) and with fluorescein as a standard. The chirality of viriplanin A is characterized by a typical CD spectrum. Since the FAB-MS did not show a definite molecular ion, an approximate molecular formula of $C_{78\sim80}H_{105\sim110}N_3O_{36\sim40}$ (MW ~ 1,700) was estimated from the elemental analysis, the UV extinctions (Table 1) compared with nogalamycin (**1a**) and the addition of structural elements seen in the ^{13}C NMR spectrum and derived by methanolysis.

The IR spectrum (Fig. 2) has typical absorptions in the region of an ester carbonyl (1715 cm^{-1}) and an α-hydroxyanthraquinone (1660, 1620 cm^{-1}).

The complex ^1H NMR spectrum (200 MHz, $CDCl_3$ - CD_3OD) of viriplanin A displayed the following main resonances (Fig. 3): δ 0.7 ~ 2.2 (11 methyl groups), 2.40 (6H, s, $N(CH_3)_2$), 3.29 (3H, s, OCH_3), 3.77 (3H, s, ester-OCH_3), 4.8 ~ 5.5 (7H, anomeric protons), 5.78 (1H, d, J = 3.2 Hz), 7.02 (1H, d, J = 1.7 Hz, olefinic proton), 7.21 and 7.50 (1H each, s, aromatic protons). These signals indicate that viriplanin A contains seven sugar moieties linked to an aglycone unit, which is very similar to nogala-

Table 1. UV absorption bands of viriplanin A and nogalamycin (**1a**) in different solvents.

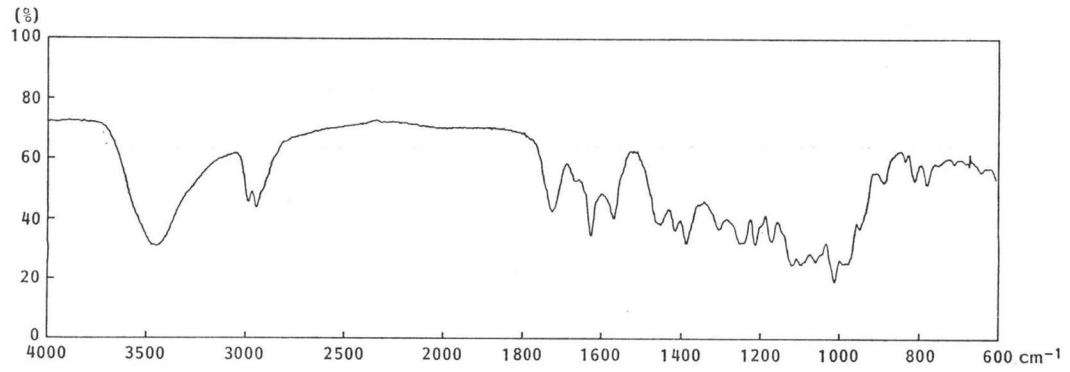
Solvent system	λ_{max} in nm ($E^{1\%}_{1cm}$)	
	Viriplanin A (MW~1,700)	Nogalamycin (MW 787)
MeOH	475 (91), 291 (47), 258 (135), 236 (299)	476 (172), 291 (106), 258 (273), 236 (612)
MeOH - HCl	470 (94), 291 (50), 258 (132), 235 (332)	471 (182), 290 (110), 258 (275), 235 (632)
MeOH - NaOH	539 (86), 328 (22), 291 (46), 239 (273)	548 (161), 327 (44), 288 (90), 239 (512)

Table 2. Rf values (TLC, silica gel) of viriplanin A and nogalamycin (**1a**).

Compounds	Solvent systems			
	1	2	3	4
Viriplanin A	0.23	0.49	0.09	0.55
Nogalamycin	0.31	0.29	0.66	0.22
Fluorescein	0.47	0.68	0.00	0.88

1; $CHCl_3$ - MeOH (80: 20), 2; $CHCl_3$ - MeOH - AcOH (80: 20: 9), 3; $CHCl_3$ - MeOH - NH_4OH (40: 6: 1), 4; BuOH - AcOH - H_2O (60: 20: 20).

Fig. 2. IR spectrum of viriplanin A in KBr.



mycin (Table 3). This conclusion is further supported by the absorptions of the ^{13}C NMR spectrum (DEPT). In addition to the 14 sp^2 carbons, the typical sp^3 carbons (C-7 to C-10, $COOCH_3$, C-1′ to C-5′ and ($N(CH_3)_2$) were assigned (Table 4). A group of eight signals at δ 96~102 arises from anomeric carbon atoms (CH) including that of C-1′. Additionally two quaternary carbon atoms were detected in this region (δ 101.5, 101.6). Neither NMR spectrum could be analyzed to the extent required to give the total structure of the antibiotic.

Viriplanin A in solution is very unstable in the light. This can be seen during successive HPLC analysis (Fig. 4). Irradiation with an incandescent lamp for 12 hours caused a total conversion of viriplanin A (peak A) giving initially unstable intermediates (peaks B and C) and ultimately a single conversion product (peak D). The very quick conversion of viriplanin A was independent of solvent, oxygen or radical scavengers. This photo-reaction is much more rapid than *N*-demethylation, which has been observed for nogalamycin[4]. If the conversion product (peak D) of viriplanin A is exposed to light for three days, further degradation products can be detected.

Fig. 3. ^1H NMR spectrum of viriplanin A in $CDCl_3$ - CD_3OD (4 : 1) at 200 MHz.

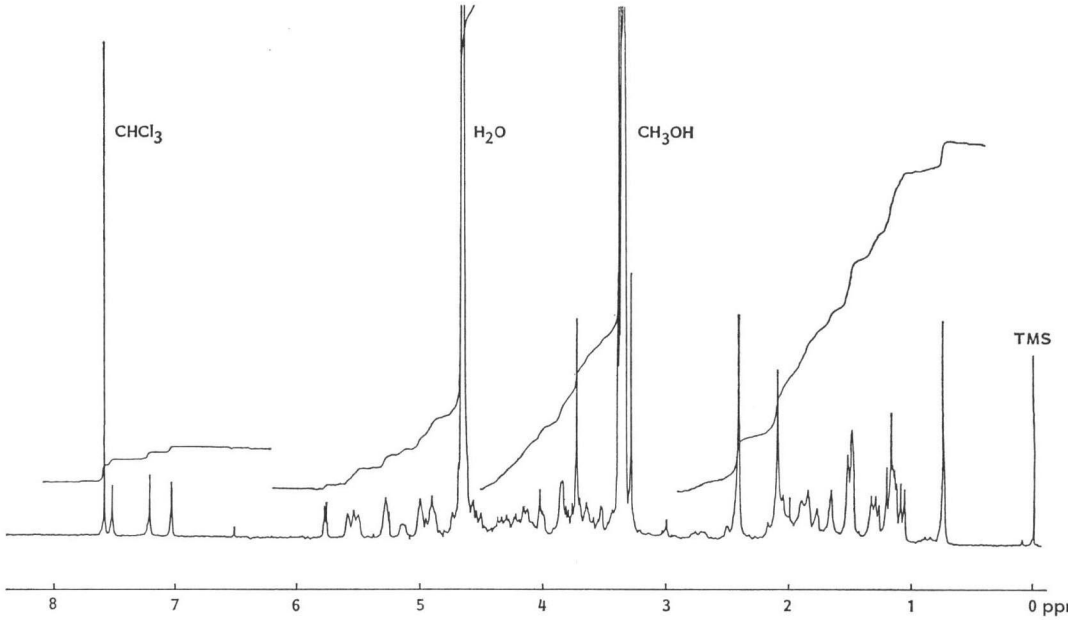


Table 3. Assignments of ^1H NMR chemical shifts δ (ppm) of viriplanin A (aglycone part), **2a**, **2b** and **3** at 200 MHz.

Proton	Viriplanin A ($CDCl_3$ - CD_3OD)	**2a** (CD_3OD)	**2b** ($CDCl_3$)	**3** ($CDCl_3$)
1′-H	5.78 d	5.85 d	6.01 d	6.03 d
2′-H	4.25 dd	4.36 dd	5.52 dd	5.55 dd
3′-H	2.70 dd	2.95 dd	2.95 dd	2.97 dd
4′-H	4.12 d	4.09 d	5.40 d	5.40 d
5′-CH_3	1.65 s	1.64 s	1.51 s	1.50 s
3-H	7.21 s	6.81 s	7.19 s	7.20 s
7-H	4.88 m	4.65 m	4.77 m	8.13 d
8-H_a	*	2.44 m	2.50 m	—
8-H_b	*	2.13 m	1.90 m	—
8-$H_{aromatic}$	—	—	—	7.56 d
10-H	4.00 s	3.76 s	4.05 s	—
11-H	7.50 s	7.24 s	7.90 s	8.61 s
9-CH_3	1.44 s	1.48 s	1.42 s	2.55 s
$COOCH_3$	3.77 s	3.64 s	3.84 s	4.11 s
7-OCH_3	—	3.53 s	3.40 s	—
$N(CH_3)_2$	2.40 s	2.62 s	2.28 s	2.26 s
Aliphatic acetyl	—	—	2.20 s	2.20 s
	—	—	2.21 s	2.22 s
Phenolic acetyl	—	—	2.42 s	2.44 s
	—	—	2.46 s	2.58 s

* Signals overlapped.

The Aglycone of Viriplanin A

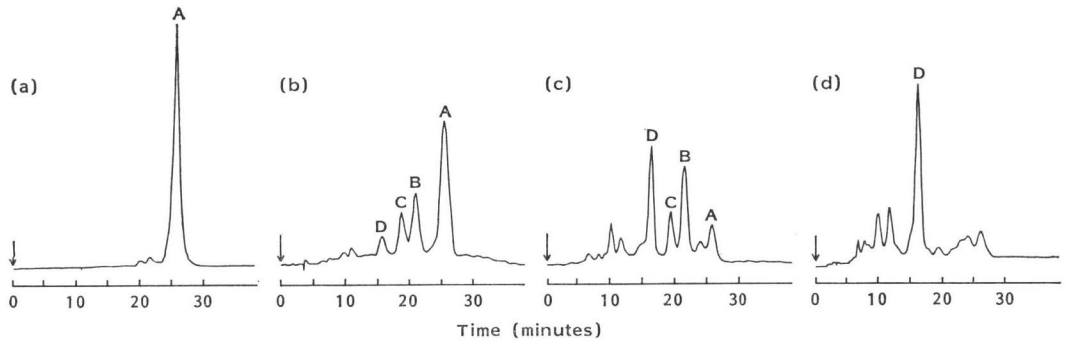
Acidic methanolysis of viriplanin A (2.5 N HCl in methanol, 6 hours, 20°C) gave the aglycone 7-*O*-methylviriplanol (**2a**) only. At elevated temperature (60°C) an additional red compound, viriplanene,

Table 4. ^{13}C Chemical shifts δ (ppm) of viriplanin A (aglycone part, without sp^2 carbon atoms) at 75.4 MHz, **1b**, **2a**, **2b** and **3** at 50.3 MHz.

Carbon	Viriplanin A (DMSO-d_6)	**1b**[3] (DMF-d_7)	**2a** (CD_3OD)	**2b** ($CDCl_3$)	**3** ($CDCl_3$)
C-7	71.6	72.5	72.9	71.3	132.3*
C-8	38.6	36.2	36.5	38.0	126.3*
C-9	69.4	69.2	70.2	69.6	131.6*
9-CH_3	29.1	30.1	30.8	29.3	21.3*
C-10	57.8	58.9	59.2	57.2	132.6*
Ester-CO	172.0	171.8	172.4	170.0	170.3
Ester-OCH_3	51.7	52.1	52.7	52.4	52.8
C-1′	96.3	97.8	98.5	93.7	93.8
C-2′	66.4	70.8	69.1	68.9	68.8
C-3′	60.8	66.6	63.0	57.4	57.4
C-4′	78.0	73.5	76.5	75.2	75.2
C-5′	76.2	76.0	77.9	75.0	75.0
5′-CH_3	22.7	24.2	22.8	22.0	21.9*
$N(CH_3)_2$	43.6	41.1	43.5	42.0	42.0
7-OCH_3	—	58.0	58.3	58.2	—

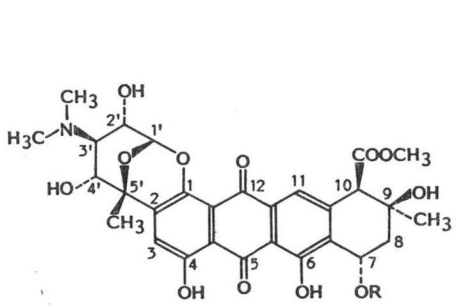
* Tentative assignments.

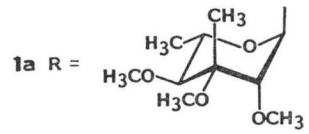
Fig. 4. HPLC analysis of a viriplanin A solution (MeOH) during irradiation with an incandescent lamp. Start (a), 3 hours (b), 6 hours (c), 12 hours (d).



was detected. These compounds could be separated by silica gel chromatography after acetylation with acetic anhydride - pyridine giving 2′,4′,4,6-*O*-tetraacetyl-7-*O*-methylviriplanol (**2b**) and 2′,4′,4,6-*O*-tetraacetylviriplanene (**3**). The molecular formula $C_{30}H_{33}NO_{12}$ of **2a** is based on the FD mass spectrum (*m/z* 599, M^+) in connection with UV extinctions and the ^1H and ^{13}C NMR data given in Tables 3 and 4. These signals indicate that **2a** is very close to 7-*O*-methylnogalarol (**1b**)[3] except for the amino sugar moiety. Its protons were unequivocally identified by selective decoupling of **3** at the resonances of the protons attached to the adjacent carbon atoms. It was found that 2′-H and 3′-H have axial configurations ($J_{2',3'} = 11.5 \sim 11.8$ Hz), whereas the configurations of 1′-H and 4′-H are equatorial ($J_{1',2'} = 3.5 \sim 4.0$ Hz, $J_{3',4'} = 3.0 \sim 3.4$ Hz). For this reason the added amino sugar moiety is a C-4′ epimer of **1b** with the uncertainty about the absolute configuration of the 3,6-dideoxy-3-dimethylaminogalactopyranose. The ^{13}C NMR spectrum of **2a** showed only one resonance at δ 98.5. In addition a group of four signals at δ 63 ~ 78 arises from carbon atoms substituted with oxygen or nitrogen as in an amino sugar. The chemical shifts at δ 191.3 (C-5) and 181.5 (C-12) indicate that only one of the anthraquinone carbonyl groups is hydrogen bonded.

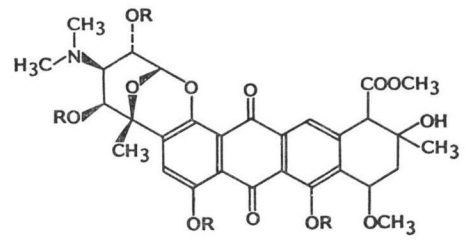
Scheme 1.





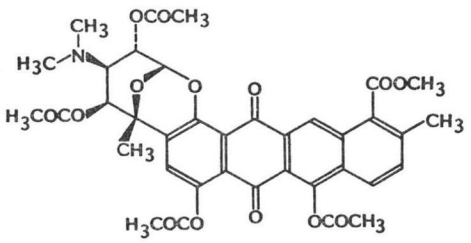
1a R =

1b R = CH_3



2a R = H

2b R = $COCH_3$



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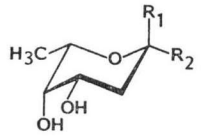
The Sugar Moieties

In addition to the chromophore unit the methanolysis mixture contained a carbohydrate fraction, which was separated by silica gel column chromatography to give the methyl glycosides of three sugars. They were identified as mixtures of the anomeric methyl glycosides of 2-deoxy-L-fucose (deFuc), decilonitrose (DEC) and the methyl ester of 4-*O*-mesaconoyl-L-diginose (MDig). ^{1}H NMR spectral analysis of the deFuc fraction indicated a mixture containing 75% methyl α-L-pyranoside (**4a**), 15% of the β-anomer (**4b**) and 10% of a furanoside. This conclusion is based upon the chemical shifts and coupling constants of the anomeric protons [1-H of **4a**: δ 4.76, $J_{1eq,2ax}$ = 3.5 Hz; 1-H of **4b**: δ 4.25, $J_{1ax,2ax}$ = 9.7 Hz; 1-H (furanoside): δ 5.10]. Weight analysis indicated that there are four molecules of deFuc in viriplanin A. Hydrolysis of the anomeric methyl glycosides of deFuc with 0.02 N H_2SO_4 at 80°C for 5 hours gave 2-deoxy-L-fucose as shown by the optical rotation[5].

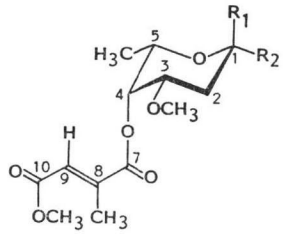
The mixture of the methyl glycosides of MDig was separated on silica gel with petroleum ether - ethyl acetate (2 : 1) into the α-pyranoside **5a**, the β-pyranoside **5b** and a furanoside **6**. The IR spectra of the oily methyl glycosides show the presence of an ester carbonyl group near 1720 cm^{-1} and an olefinic double bond near 1645 cm^{-1}. The EI mass spectra of **5a** and **5b** demonstrate typical fragments of methyl pyranosides arising from the loss of a methoxyl radical (*m/z* 271), the degradation of the pyranose ring giving a C-3/C-4 fragment with the attached substituents (*m/z* 200) and the loss of the mesaconoyl residue (*m/z* 127, base peak). This peak is also seen in the EI mass spectrum of the furanoside. More typical here is the fragment given by the loss of the whole side chain at C-4 of the tetrahydrofuran ring (*m/z* 131) giving the ion *m/z* 99 by further loss of methanol. Cleavage of the five membered ring gives the peak at *m/z* 102 (C-1 to C-3). The base peak (*m/z* 71) results by the loss of methoxyl from the latter ion.

Acylation of the pyranoside arising from the mesaconoyl residue results in a significant downfield

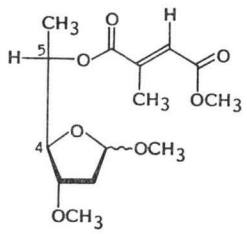
Scheme 2.



4a $R_1 = OCH_3$, $R_2 = H$
4b $R_1 = H$, $R_2 = OCH_3$



5a $R_1 = OCH_3$, $R_2 = H$
5b $R_1 = H$, $R_2 = OCH_3$



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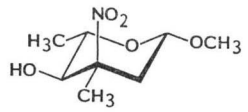

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Table 5. ^1H NMR chemcial shifts δ (ppm) and coupling constants J (Hz) of the methyl glycosides isolated from viriplanin A in $CDCl_3$ at 200 MHz.

Proton	4a	5a	5b	6	7	Furanoside of 7
1-H	4.76 dd	4.88 dd	4.40 dd	5.08 dd	4.52 dd	5.02 dd
2-H_{ax}	1.7~1.8 m	1.8~2.2 m	1.75 ddd	2.11 ddd	1.75 dd	2.32 dd
2-H_{eq}	1.8~1.9 m	1.8~2.2 m	2.06 ddd	2.02 ddd	2.75 dd	2.84 dd
3-H	4.00 m	3.73 m	3.44 m	3.68 m	—	—
3-CH_3	—	—	—	—	1.74 s	1.73 s
4-H	3.60 m	5.34 dd	5.28 dd	4.08 dd	3.3~3.4 m	4.50 m
5-H	3.90 m	3.98 m	3.65 m	5.16 m	3.70 m	3.85 m
5-CH_3	1.25 d	1.16 d	1.23 d	1.33 d	1.38 d	1.37 d
8-CH_3	—	2.35 d	2.33 d	2.30 d	—	—
9-H	—	6.86 d	6.85 d	6.77 d	—	—
1-OCH_3	3.30 s	3.33 s*	3.53 s	3.33 s	3.48 s	3.37 s
3-OCH_3	—	3.34 s*	3.35 s	3.40 s	—	—
Ester-OCH_3	—	3.78 s	3.77 s	3.78 s	—	—
J (Hz)						
$J_{1,2ax}$	3.5	3.0	9.7	5.5	9.2	5.5
$J_{1,2eq}$	<1	<1	2.0	0.8	2.0	2.5
J_{2gem}	13.5	13.0	12.4	14.0	14.8	14.5
$J_{2ax,3}$	11.5	11.4	10.0	7.7	—	—
$J_{2eq,3}$	5.5	5.6	5.5	2.4	—	—
$J_{3,4}$	2.5	<3	<1	4.4	—	—
$J_{4,5}$	<2	<3	3.0	4.4	9.5	8.5
J_{5,CH_3}	6.5	6.6	6.5	6.4	6.0	6.2
$^4J_{trans}$	—	1.7	1.6	1.5	—	—

* Values interchangeable.

shift of 4-H (δ 5.34 in **5a**) compared with 3-H (δ 3.73), which is only influenced by the methoxyl group of diginose (Dig). In the furanoside **6** the mesaconoyl residue seems to be linked at C-5 (5-H: δ 5.16), while 4-H (δ 4.08) and 3-H (δ 3.68) show the normal position. The migration of acyl residues in 2,6-

dideoxypyranoses yielding a furanose is well-known, because both isomers have similar energy contents[6].

The carbonyl function of the mesaconic acid in the antibiotic viriplanin A seems to be free. There is only one ester methoxyl group present (δ 3.77), which should belong to the aglycone. To determine the position of the methyl group of the mesaconoyl residue the α-pyranoside **5a** was cleaved by ozonolysis. The IR spectrum of the isolated product showed two carbonyl groups (1730, 1755 cm^{-1}), the ^1H NMR spectrum showed a methyl group as a singlet at δ 2.51. These data indicate that the mesaconic acid residue had been converted into a pyruvic acid residue. For this reason the position of the methyl group of the mesaconic acid unit must be near the CO-group linked to the sugar moieties. The optical rotation of the α-pyranoside **5a** ($[\alpha]_D^{20}$ $-110.7°$ (*c* 1, $CHCl_3$)) compared with the β-pyranoside **5b** ($[\alpha]_D^{20}$ $+11.1°$ (*c* 1, $CHCl_3$)) demonstrates that diginose belongs to the L-series (HUDSON isorotation rule)[7].

The sugar mixture obtained by methanolysis contained methyl glycosides of a third carbohydrate moiety, which proved to be identical with the recently published nitro sugar decilonitrose[8] (DEC). The nitro sugar was isolated as an anomeric mixture of a methyl-β-pyranoside **7** and a furanoside. The mixture is an unstable colorless syrupy solid, which easily sublimes under reduced pressure. Its EI mass spectrum showed the ion $(M-NO_2)^+$ at *m/z* 159. The nitro group gives a typical absorption at 1540 cm^{-1} in the IR spectrum.

Biological Activity

In the disc-diffusion assay, carried out in red light, viriplanin A inhibits the growth of Gram-positive bacteria such as *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* and *Micrococcus lysodeikticus*. The MIC values range from 0.1 to 1 μg/ml. Viriplanin A shows no activity against yeasts and Gram-negative bacteria (*Escherichia coli* ATCC 9637, *Pseudomonas aeruginosa*, *Proteus vulgaris* OX 19). In the *in vivo* test against leukemia P388 in mice the antibiotic is toxic at a dose of 0.5 mg/kg, while no effect was observed at lower doses down to 0.02 mg/kg. In this test no difference was found between purified viriplanin A and the raw viriplanin mixture. The acute toxicity (LD_{50}) of raw viriplanin in mice has been evaluated for different ways of administration: po (100 mg/kg)>ip (2~3 mg/kg) >iv (1~2 mg/kg)>sc (1 mg/kg).

The antiviral activity of raw viriplanin was determined in a plaque reduction assay in murine L-929 fibroblasts against Herpes simplex virus type 1[9]. A complete inhibition of plaque formation was observed at 0.1~0.01 μg/ml; concentrations at 1 μg/ml and above caused cytotoxicity. A significant reduction of the virus titer was found in a titer reduction assay in Vero cells at 1 μg/ml[10]. Purified viriplanin A completely lacked antiviral activity. The cytotoxic concentration was 0.1 μg/ml in assays with Vero cells; no antiviral activity was obtained by further dilution. These results cannot be interpreted unequivocally. The isolation and characterization of the remaining components from raw viriplanin as well as the purification of viriplanin D (peak D) may help to explain this surprising effect.

Discussion

Viriplanin A belongs to a group of new anthracycline antibiotics with an aglycone similar to that of nogalamycin (**1a**) and with sugar moieties containing nitro groups. Decilorubicin[11] and arugomycin[12] are the first examples of these rather complex antibiotics. The aglycone of viriplanin A seems to be identical with that of arugomycin, the differences are situated in the sugar part of the molecules[13,14]. The elemental analysis (N 2.43%) indicates three nitrogens in viriplanin A, and there is only one carbohydrate methoxyl group in the NMR spectra corresponding to only one diginose unit in the antibiotic. In this and in the dicarboxylic acid residue (mesaconate instead of fumarate) viri-

planin A differs remarkably from arugomycin. Viriplanin A therefore is a new antibiotic.

Comparing the NMR spectra of viriplanin A with those of methyl decilonitroside there is a striking inconsistency in the ^1H NMR singlet of 3-CH_3 and the ^{13}C NMR signal of C-3 in free decilonitroside[8,12,13] (δ_H 1.74; δ_C 89.6) compared with viriplanin A (δ_H 0.75, two methyl groups; δ_C 101.5 and 101.6). The typical decilonitroside signals are present in arugomycin, they are missing in viriplanin A. From these results we concluded i) viriplanin A contains two sugar moieties similar to decilonitrose and ii) methyl β-decilonitroside isolated from viriplanin A is an artefact of methanolysis. In addition the typical IR absorption band at 1545 cm^{-1} present in the spectra of decilonitroside, decilorubicin[15] and arugomycin as well is not detectable in the viriplanin A spectrum (Fig. 2). The nature of the nitro sugar within the parent compound viriplanin A as well as the sequence of the oligosaccharide and its linkage to the aglycone are under investigation.

Experimental

General

Melting points were determined on a Reichert hot-stage microscope and are uncorrected. IR spectra were recorded on a Perkin Elmer Model 298 spectrometer and UV spectra on a Zeiss DMR 21 spectrophotometer. Optical rotations were taken with a Perkin Elmer model 241 polarimeter. All CD spectra were obtained on a Jasco J-500 A spectropolarimeter in MeOH - sodium phosphate buffer pH 7.2 (65 : 35). ^{13}C and ^1H NMR spectra were measured on a Varian XL-200 spectrometer operating at 4.7 Tesla. Chemical shifts are expressed in δ values (ppm) with TMS as an internal standard. The multiplicity of the ^{13}C NMR values were assigned by APT or DEPT techniques. The mass spectra were taken by a Varian MAT 311 A (EI) and a Finnigan MAT 8230 (FD) mass spectrometer.

Analyticals

Thin-layer chromatography (TLC) was carried out on silica gel plates (Macherey & Nagel SIL G/UV 254+366, 0.25 mm silica gel on glass), column chromatography on Silica gel 60 (0.08 mm, Macherey & Nagel). The amount of viriplanin A was determined by HPLC with a Waters 6000 A Solvent Delivery System and a Waters 450 Variable Wavelength Detector using a 5 μm Nucleosil RP-18 column (4×300 mm) developed with a mobile phase of MeOH - aq phosphate buffer pH 7.20 (65: 35) at a flow rate of 0.7 ml/minute. Viriplanin A was detected by UV absorption at 470 nm.

Fermentation Studies

Ampullariella regularis (strain SE 47) was cultured on agar slants composed of sucrose 3%, Bacto-peptone (Difco) 0.25%, hydrolyzed casein, acidic (Oxoid) 0.25%, $K_2HPO_4 \cdot 3H_2O$ 0.1%, KCl 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $FeSO_4$ 0.01% and agar (Difco) 2%. After an incubation time of 10 days at 20°C, the sporangia were suspended in 15 ml of a sterile 0.9% aq NaCl solution. Three 1-liter Erlenmeyer flasks containing each 140 ml of sterile medium of soybean meal 3%, glycerol 3% and $CaCO_3$ 0.3% were inoculated with 1 ml of the sporangia suspension. Fermentation on a rotary shaker was allowed to proceed for four days at 24°C. These cultures were used as inoculum for three glass fermentors containing 8 liters of sterile medium composed of sucrose 2%, yeast extract 1.5%, glucose 0.5%, $CaCO_3$ 0.2%, K_2HPO_4 0.1%, NaCl 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05% and $FeSO_4$ 0.001%. Agitation; 400 rpm, aeration, 4 liters/minute, pH 7.8~8.0. After 65 hours of cultivation at 28°C, the culture broths were combined and the mixture centrifuged at 3,000 rpm. The mycelium was extracted with 18 liters MeOH and removed by centrifugation. The methanolic extract was evaporated to a small volume, which was diluted with 400 ml H_2O and extracted 15 times with butanol. The combined extracts were concentrated under reduced pressure yielding 6.65 g of a crude product. The culture filtrate (18 liters) was adjusted to pH 7.0 with 1 N H_2SO_4 and extracted twice with 9 liters butanol. The extracts were concentrated *in vacuo* to a volume of 3 liters yielding a red precipitate of crude product (15 g). The crude products were washed with CCl_4 and dried *in vacuo* at room temp.

Isolation of Viriplanin A

The crude antibiotic was dissolved in $CHCl_3$ - MeOH - 33% NH_4OH (120: 30: 1) and separated from

insoluble material. The filtrate and washing were combined and concentrated *in vacuo*. Purification was carried out by chromatography on a 100×6 cm column of silica gel eluting with $CHCl_3$ - MeOH - 33% NH_4OH (95: 5: 1 and 120: 30: 1 stepwise). 6.3 g raw viriplanin mixture was isolated from the main zone. The raw antibiotic was divided into portions of 700 mg for further purification by chromatography on a pre-packed RP-8 silica gel column (Lobar size C, Merck). Elution was done with MeOH - 0.01 M aq phosphate buffer pH 7.2 (65 : 35) at a flow rate of 130 ml/hour. Viriplanin A was eluted after 14 hours to yield 114 mg, which was desalted on Sephadex-LH 20 (column: 40×2.5 cm, MeOH). Analytical HPLC (parameters given above) demonstrated a purity of about 95% for viriplanin A: MP>210°C (dec); $[\alpha]_D^{20}$ +144° (*c* 0.075, $CHCl_3$ - MeOH, 9: 1); Rf values see Table 2; IR (KBr, Fig. 2) 1715, 1660 (sh), 1620, 1565 cm^{-1}; UV see Table 1; ^1H NMR (200 MHz, $CDCl_3$ - CD_3OD) see Fig. 3 and Table 3; ^{13}C NMR (75.4 MHz, DMSO-d_6) values completing Table 4, δ 13.7, 16.3, 16.4, 16.6, 17.2, 18.2, 18.7, 31.1, 32.6, 33.5, 34.0, 41.2, 41.3, 55.3, 63.9, 64.5, 65.3, 67.0, 67.5, 67.6, 68.3, 69.9, 70.9, 71.4, 72.4, 80.4, 80.9, 81.2, 86.4, 86.8, 98.5, 98.7, 99.0, 100.4, 100.6, 100.7, 101.5, 101.6, 114.4, 115.1, 117.1, 121.6, 130.2, 132.2, 133.5, 134.2, 137.0, 146.1, 155.4, 167.5, 168.7, 179.5, 180.1; CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{23}\times10^{-4}$) 476 (+1.2), 360 (+1.5), 315 (+0.9), 287 (−2.9).

Anal Calcd for $C_{78\sim80}H_{105\sim110}N_3O_{36\sim40}$: C 54.1~57.9, H 6.1~6.6, N 2.4~2.5.
Found: C 54.84, H 6.19, N 2.43.

Photolytic Studies of Viriplanin A

The photolytic degradation reactions of viriplanin A were run under comparable conditions. 2 mg of viriplanin A was dissolved in 5 ml of solvents such as MeOH, MeOH - 0.05% aq phosphate buffer pH 7.2 (7 : 3), MeOH - 0.02% hydroquinone. The solutions were transferred into 5 ml glass flasks and exposed to an incandescent lamp (60 W, 30 cm distance) for 12 hours. These experiments were repeated under argon atmosphere.

Methanolyses of Raw Viriplanin

[a]: A solution of raw viriplanin (2.5 g) in 2.5 N methanolic hydrogen chloride (100 ml) was stirred at room temp for 6 hours in a darkened glass flask. The mixture was neutralized with sodium bicarbonate and extracted twice with EtOAc. The extracts were evaporated to dryness and the residue was chromatographed on silica gel (column: 20×4 cm, $CHCl_3$ - MeOH, 4: 1) to give a mixture of methyl glycosides (410 mg) and a red solid of 7-*O*-methylviriplanol (**2a**, 135 mg). The methyl glycosides were separated on a silica gel column (20×5 cm, $CHCl_3$ - MeOH, 9: 1) into the anomeric methyl glycosides of 2-deoxy-L-fucose (**4a**/**4b**, 250 mg) and a complex carbohydrate fraction, from which methyl decilonitroside could be isolated by silica gel chromatography (column: 15×2.5 cm, $CHCl_3$ - acetone, 9: 1) as an anomeric mixture (3 mg) of the β-pyranoside **7** and a furanoside.

2a: MP>200°C (dec); $[\alpha]_D^{20}$ +350° (*c* 0.01, MeOH); IR (KBr) 1730, 1660, 1620 cm^{-1}; UV λ_{max}^{MeOH}nm (ε) 476 (13,100), 290 (sh, 8,800), 252 (22,900), 236 (36,600); ^1H NMR (200 MHz, CD_3OD) see Table 3; ^{13}C NMR (50.3 MHz, CD_3OD) values completing Table 4, δ 114.5 (C-5a), 115.3 (C-12a), 117.4 (C-4a), 120.0 (C-11), 123.9 (C-3), 132.2 (C-6a), 134.1 (C-11a), 140.5 (C-2), 144.2 (C-10a), 148.8 (C-1), 157.1 (C-4), 162.8 (C-6), 181.5 (C-12), 191.3 (C-5); FD-MS *m/z* 599 (M^+).

Anal Calcd for $C_{30}H_{33}NO_{12}$: C 60.10, H 5.51, N 2.34.
Found: C 58.80, H 5.84, N 2.48.

4a: Rf 0.44 ($CHCl_3$ - MeOH, 4: 1); ^1H NMR (200 MHz, $CDCl_3$) see Table 5; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 16.7 (5-CH_3), 32.6 (C-2), 54.8 (1-OCH_3), 65.4 (C-3), 70.5 (C-5), 71.0 (C-4), 98.6 (C-1); MS (70 eV) *m/z* (abundant) 131 (10%, M−OCH_3), 118 (8%), 113 (9%), 104 (25%), 99 (3%), 87 (4%), 73 (7%), 71 (8%), 60 (94%), 59 (100%).

7: Rf 0.60 (toluene - EtOAc, 1: 1); IR (film) 1540 cm^{-1} (NO_2); ^1H NMR (200 MHz, $CDCl_3$) see Table 5; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 18.3 (5-CH_3), 25.2 (3-CH_3), 41.6 (C-2), 56.6 (1-OCH_3), 71.0 (C-5), 77.2 (C-4), 89.5 (C-3), 98.5 (C-1); MS (70 eV) *m/z* (abundant) 159 (1%, M−NO_2), 126 (3%), 115 (22%), 99 (16%), 83 (100%), 71 (90%).

[b]: 5 g raw viriplanin were dissolved in 100 ml of 2.5 N methanolic hydrogen chloride. The mixture was stirred at 60°C for 1 hour and then at room temp for 16 hours. The work-up procedure was done as described in [a] yielding 500 mg of **4a**/**4b** and a mixture of anomeric methyl glycosides of

4-*O*-mesaconoyl-L-diginose methyl ester. Those were separated on silica gel (column: 15×2.5 cm, hexane - EtOAc, 2: 1) into the α-pyranoside **5a** (50 mg), the β-pyranoside **5b** (50 mg) and a furanoside **6** (20 mg). The chromophore residue in the organic layer (210 mg) was composed of **2a** and viriplanene.

5a: Colorless syrupy oil; $[\alpha]_D^{20}$ −110.7° (*c* 1, $CHCl_3$); Rf 0.30 (hexane - EtOAc, 2: 1); IR (film) 1720, 1645 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) see Table 5; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.5 (8-CH_3), 16.9 (5-CH_3), 31.2 (C-2), 51.6 (10-OCH_3), 54.9 (1-OCH_3), 56.3 (3-OCH_3), 64.9 (C-5), 70.2 (C-4), 73.6 (C-3), 98.9 (C-1), 126.7 (C-9), 143.8 (C-8), 166.3 (C-10), 167.0 (C-7); MS (70 eV) *m/z* (abundant) 302 (3%, M^+), 271 (8%), 200 (32%), 127 (100%), 99 (36%), 75 (85%), 59 (23%).

5b: Colorless syrupy oil; Rf 0.21 (hexane - EtOAc, 2: 1); $[\alpha]_D^{20}$ +11.1° (*c* 1, $CHCl_3$); IR (film) 1725, 1650 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) see Table 5; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.5 (8-CH_3), 16.8 (5-CH_3), 32.9 (C-2), 51.5 (10-OCH_3), 56.5 (1-OCH_3), 56.6 (3-OCH_3), 69.1, 69.5 (C-3, C-4, C-5), 101.3 (C-1), 126.9 (C-9), 143.6 (C-8), 166.3 (C-10), 167.0 (C-7); MS (70 eV) *m/z* (abundant) 302 (0.4%, M^+), 271 (2%, $M-OCH_3$), 200 (20%), 127 (100%), 99 (99%), 75 (27%), 59 (11%).

6: Colorless syrupy oil; Rf 0.24 (hexane - EtOAc, 2: 1); IR (film) 1720, 1645 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) see Table 5; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.4 (8-CH_3), 16.1 (5-CH_3), 38.6 (C-2), 51.6 (10-OCH_3), 55.1 (1-OCH_3), 57.5 (3-OCH_3), 71.8 (C-3), 81.5, 84.3 (C-4, C-5), 105.1 (C-1), 126.5 (C-9), 144.0 (C-8), 166.3 (C-10), 166.5 (C-7); MS (70 eV) *m/z* (abundant) 271 (5%, $M-OCH_3$), 200 (6%), 131 (47%), 127 (56%), 113 (13%), 102 (70%), 101 (23%), 99 (82%), 95 (36%), 71 (100%).

2-Deoxy-L-fucose (DeFuc)

100 mg of the anomeric methyl glycosides **4a**/**4b** were treated with 75 ml 0.02 N aq H_2SO_4 at 80°C for 5 hours. The H_2SO_4 was neutralized with a satd solution of $Ba(OH)_2$ in H_2O, the precipitated $BaSO_4$ was removed by centrifugation. After removal of the solvent, the remaining syrup was dissolved in 2 ml acetone. DeFuc precipitated as colorless, amorphous solid (70 mg) after a few days at 0°C: MP 100~103°C; $[\alpha]_D^{20}$ −56.8° (*c* 0.94, MeOH), −132.7° (*c* 1.07, acetone); ^1H NMR (200 MHz, CD_3OD) δ 1.19, 1.25 (d, J=6.5 Hz, 5-CH_3, α-deFuc, β-deFuc), 1.6~1.8 (m, 2-H_{ax}), 1.8~2.0 (m, 2-H_{eq}), 3.54 (dd, J=1/2 Hz, 4-H), 4.00 (m, 3-H), 4.10 (dq, J=1~6.5 Hz, 5-H), 4.68 (dd, J=2.2~9.7 Hz, 1-H, β-deFuc), 5.25 (dd, J=1~3.5 Hz, 1-H, α-deFuc).

Ozonolysis of **5a**

A solution of 20 mg **5a** in 20 ml MeOH was poured into a reaction vessel and cooled to −78°C. A gas stream of 3.3 g ozone/hour (generated by a Fischer model 520 ozone generator) was conducted through the reaction vessel and a washing flask containing an aq potassium bromide solution. The reaction was allowed to proceed for about 2 minutes, until KBr was seen to be oxidized to Br_2. The reaction solution was rinsed with nitrogen for 2 minutes and then treated with 10 ml dimethylsulfide. The mixture was stirred at 0°C for 1 hour and at room temp for 3 hours. The solvents were evaporated *in vacuo* and the residue was separated on silica gel (column: 15×2.5 cm) with $CHCl_3$ - MeOH (9: 1). Yielding 6.3 mg of methyl 4-*O*-pyruvoyl-L-diginoside: Colorless syrupy oil; IR (film) 1755, 1730 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.19 (d, J=6 Hz, 5-CH_3), 1.9~2.0 (m, 2-H, 2-H), 2.51 (s, 8-CH_3), 3.34 (s, 1-OCH_3), 3.36 (s, 3-OCH_3), 3.76 (m, 3-H), 4.02 (m, 5-H), 4.88 (dd, 1-H), 5.33 (dd, 4-H).

Acetylation of the Chromophore Fraction

146 mg of the chromophore residue of methanolysis [b] were treated with 10 ml acetic anhydride - pyridine (1: 1) and stirred at 50°C for 12 hours. The mixture was poured into 20 ml ice water and neutralized with a satd solution of $NaHCO_3$ in H_2O. The acetates were extracted with 200 ml $CHCl_3$ and the extracts evaporated to dryness. The residue (165 mg) was chromatographed on silica gel (column: 20×2.5 cm) with $CHCl_3$ - dioxane (9 : 1). The eluate of the faster zone contained 48 mg of pure 2′,4′,4,6-*O*-tetraacetylviriplanene (**3**), the slower zone was purified on silica gel (column: 20× 2.5 cm) with $CHCl_3$ - MeOH (9: 1) as solvent yielding 33 mg of 2′,4′,4,6-*O*-tetraacetyl-7-*O*-methyl-viriplanol (**2b**).

2b: Yellow, amorphous powder; mp 150~155°C; Rf 0.25 ($CHCl_3$ - dioxane, 9 : 1); IR (KBr) 1775, 1745, 1675, 1595 cm^{-1}; UV λ_{max}^{MeOH} nm (ε) 383 (6,700), 262 (29,800); ^1H NMR (200 MHz, $CDCl_3$) see Table 3; ^{13}C NMR (50.3 MHz, $CDCl_3$) values completing Table 4, δ 20.9 (q), 21.1 (q), 21.2 (q) 21.3 (q,

4-acetyl-CH_3), 112.6 (d), 120.6 (s, C-4a), 126.5 (d), 126.9 (s), 127.0 (d), 132.4 (s), 134.0 (s), 135.2 (s, C-2), 141.5 (s), 142.5 (s, C-1, C-10a), 148.3 (s), 150.0 (s, C-4, C-6), 170.4 (s), 170.5 (s), 171.3 (s, 4-acetyl-CO), 180.4 (s), 180.6 (s, C-5, C-12); MS (72 eV) *m/z* (abundant) 767 (11%, M^+), 708 (12%, M−59), 666 (4%), 611 (4%), 551 (4%), 158 (100%), 116 (78%), 87 (23%).

3: Yellow, amorphous powder; mp 155~165°C; Rf 0.38 ($CHCl_3$ - dioxane, 9: 1); IR (KBr) 1765, 1748, 1735, 1678 cm^{-1}; UV λ_{max}^{MeOH} nm (ε) 395 (9,600), 298 (22,300), 240 (49,400); ^1H NMR (200 MHz, $CDCl_3$) see Table 3; ^{13}C NMR (50.3 MHz, $CDCl_3$) values completing Table 4, δ 20.5 (q), 21.0 (q), 21.1 (q), 21.3 (q, 4-acetyl-CH_3), 108.3 (s, C-5a), 119.4 (s, C-12a), 121.7 (s, C-4a), 123.6 (d, C-11), 124.8 (d, C-3), 126.3 (d, C-8), 127.7 (s), 128.2 (s), 132.3 (s, C-2), 138.4 (s, C-10a), 142.6 (s, C-1), 147.4 (s, C-4), 150.0 (s, C-6), 168.6 (s), 168.9 (s), 169.6 (s), 170.5 (s, 4-acetyl-CO), 180.6 (s), 180.8 (s, C-5, C-12); MS (70 eV) *m/z* (abundant) 717 (4%, M^+), 658 (5%, M−59), 616 (3%), 501 (2%), 158 (100%), 116 (74%), 87 (30%).

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