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THE STRUCTURE OF OLEFICIN, A NEW POLYENIC ANTIBIOTIC EXHIBITING ANTIBACTERIAL ACTIVITY

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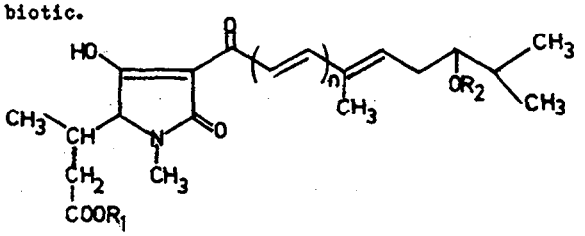
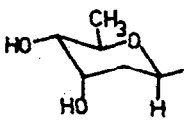
In a previous paper /1/ the isolation and partial characterization of a new antibiotic, named oleficin have been reported. Based upon its characteristics oleficin has been related to various antibiotics of known structure /1/. Closer similarity was found, however, to erythroskyrine, a pigment of Penicillium islandicum SOPP /2-4/. Direct comparison of erythroskyrine * with oleficin confirmed their similarity regarding UV, IR and NMR spectra but it also showed that the two compounds are of substantially different structures. Last year the isolation and partial characterization of α-lipomycin were reported /5/. There is a close analogy between oleficin and α-lipomycin in many respects but they are not identical, as shown by direct comparison /5/.

In this paper we report the structure elucidation of oleficin based on spectroscopic and chemical evidence, which has resulted in assuming structure I for this new antibiotic.





-β-D-digitoxoside

I R_1= H ; R_2= β-D-digitoxoside ; n= 5

II R_1= CH_3 ; R_2= H ; n= 5

V R_1= H ; R_2= β-D-digitoxoside ; n= 4

* A sample of erythroskyrine was kindly supplied by Prof. S. Shibata in 1971.

The crude oleficin, obtained according to Ref. /1/, was fractionated on a column of silicic acid with benzene-methanol. The fraction containing oleficin was further purified on Sephadex LH-20 column to yield compound I, an amorphous dark-red solid melting at 96-98 °C ; $/\alpha/_D^{30} = -182° \pm 10°$ (c 0,2 ethanol) from the ORD curve * ; (Found: C, 66,15 ; H, 7,84 ; N, 2,52 , $C_{34}H_{47}NO_9$ requires: C, 66,70 ; H, 7,7 ; N, 2,30 %). Upon TLC (silicagel-G, impregnated with 0,1 N oxalic acid ; benzene:ether:acetone = = 9:9:2 solvent) compound I gave one spot (R_f= 0,63). The main maximum of the UV spectrum ** of I was found at λ= 418,5 nm with log ε= 4,80 (both 5 and 15 mcg/ml in 96 % ethanol). Other spectroscopic data will be given later in the text.

In order to facilitate its structure elucidation, oleficin was hydrolysed with methanolic 0,1 N sulphuric acid yielding the methyl-ester of its aglycone and a mixture containing the methyl-glycosides of the sugar-portion.

Identification of the sugar-portion: The methyl-β-glycoside was separated from the above mixture (column-chromatography on silica, followed by Sephadex LH-20). It could be presumed upon analogy with α-lipomycin /5/ that the sugar involved in oleficin might be digitoxose, too. Accordingly the authentic methyl-β-D-digitoxoside was prepared by similar methods from digitoxin. The two glycosides were identical in every respect, e.g. they gave the same NMR spectra *** which also corresponded to the NMR-data reported in Ref. /5/. The D-configuration of the glycoside was confirmed by $/\alpha/_D^{25} = -24°$ (c 1,0 methanol). The splitting of the anomeric proton at δ= 4,8 ppm in the NMR spectrum of oleficin (J_{aa}= 9 Hz ; J_{ae}= 3 Hz) reveals the presence of a β-glycosidic bond. Consequently the sugar-portion of oleficin has been identified as being -β-D-digitoxoside.

Structure elucidation of the aglycone part: The crude aglycone-methyl-ester was chromatographed on a Sephadex LH-20 column with methanol to yield compound II, a dark purple solid melting at 60 °C. Upon TLC (vide supra) compound II gave one spot (R_f= 0,85).

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* For this measurement we are indebted to Dr M. Kajtár.

** UV spectra were recorded with a Unicam SP-700 spectrophotometer.

*** NMR spectra were recorded at 60 MHz on a Varian A-60D spectrometer in $CDCl_3$, using TMS as internal standard.

1. Facts assuring that compound II correctly represents the aglycone-moiety of oleficin (apart from being esterified) :

a) the chromophor remained unchanged as shown by the UV spectrum of II (λ_{max}= = 415,5 nm ; log ε= 4,80 ; 15 mcg/ml in 96 % ethanol) ;

b) the IR spectrum * of II shows only the following changes when compared with that of oleficin: $\nu_{C=O}$ = 1715 cm^{-1} (acid ; shoulder) of I is shifted to $\nu_{C=O}$ = 1730 cm^{-1} (ester) in II, ν_{C-O} = 1070 cm^{-1} of I is considerably decreased in the spectrum of II due to the absence of the sugar-portion ;

c) by superposing the NMR spectra of compound II and of methyl-β-D-digitoxoside (omitting, of course, the OCH_3 signals) the NMR spectrum of oleficin is obtained.

2. The structure of the substituted pyrroline ring of oleficin-aglycone-methyl-ester:

a) It became apparent from spectroscopic data, that similarly to erythroskyrine /3/, a β-diketone-system is involved in this ring ; IR: $\nu_{HO-C=C-C=O}$ = 1610 cm^{-1} (vs), UV of compound III (see later): λ_{max} = 244,5 and 285 nm log ε= 4,088 and 4,142, respectively (20 mcg/ml in 96 % ethanol) , red $FeCl_3$-reaction of compound III.
Similarly, the presence of N-methyl-lactam group was also confirmed by IR: $\nu_{C=O}$ = = 1690 cm^{-1} and NMR: δ $N-CH_3$ (see Table 1) of compounds I, II and III.

b) Mass spectral data ** of compound II and its hydrogenated derivative III (III was obtained by hydrogenation with Pd/ C in methanol, followed by column-chromatography on Sephadex LH-20: oil ; $/\alpha/_D^{25}$= + 25 °, c 0,1 methanol) are in agreement with these assignments and in addition furnish informations about the structure of the side chain at position-5 of the ring.
Prominent ions, which confirm the structure of the substituted pyrroline ring are depicted in Scheme 1.
The depicted arrangement of the side chain was corroborated by the NMR spectra of I, II and III as shown by the data listed in columns 1 and 2 of Table 1.

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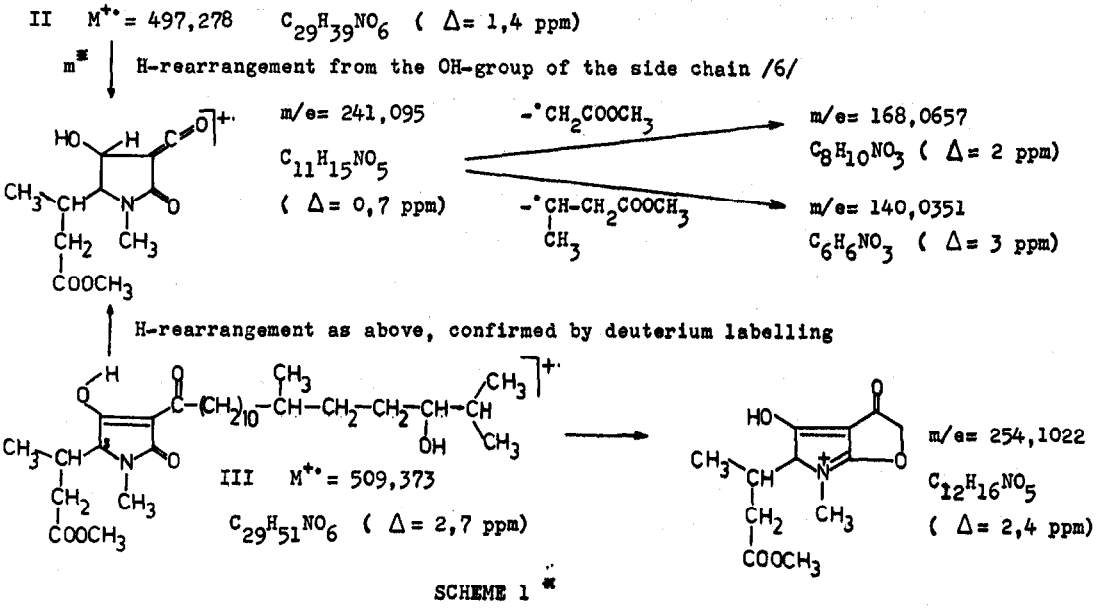
* IR spectra were recorded on a Perkin-Elmer 457 spectrometer in KBr pellets.

** Mass spectra were taken on a Varian MAT SM-1 instrument at 70 eV and R= 1250.



SCHEME 1 *

TABLE 1: SELECTED NMR DATA OF COMPOUNDS I, II AND III

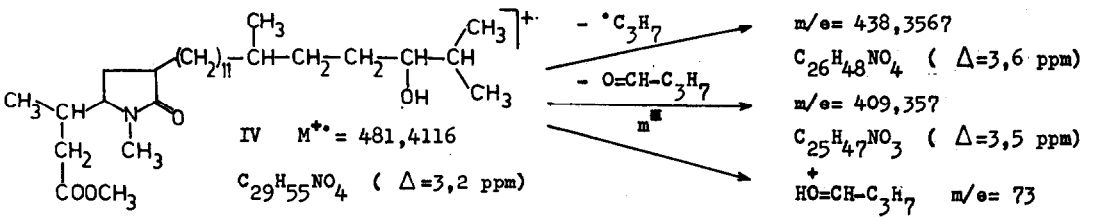
Compounds	Chemical shifts (in ppm)			
	δ C-CH_3	δ CH_2COOR	δ =C-CH_3	δ N-CH_3
I	~1; m; 9H 1,3; d; 3H; J=6 Hz (from sugar-part)	~2,4; broad	1,82; s	3,0; s
II	~1; 2d; 9H; J=6 Hz	2,3; d; J=3,5 Hz	1,82; s	3,0; s
III	0,95-1,3; m; 12H	2,3; d; J=3,5 Hz	----	3,0; s

s= singlet; d= dublet; m= multiplet; Signals, where no H-number is given, are in overlapping with other protons.

3. Structure of the aliphatic end of the polyenic side chain:

Mass spectra of compounds II, III and IV (IV was obtained by hydrogenation of III with Pt/ C in AcOH /7/, followed by preparative TLC; no IR absorption band at 1610 cm^{-1}) show prominent ions at M - $\cdot C_3H_7$, M - O=CH-C_3H_7 and m/e= 73, indicating the structure of this part of the molecule as depicted for compound IV in Scheme 2.

* High resolution mass measurements were made at R= 12 500 using PFK as reference standard. Metastable transitions (m^*) were measured by the defocussing technique.

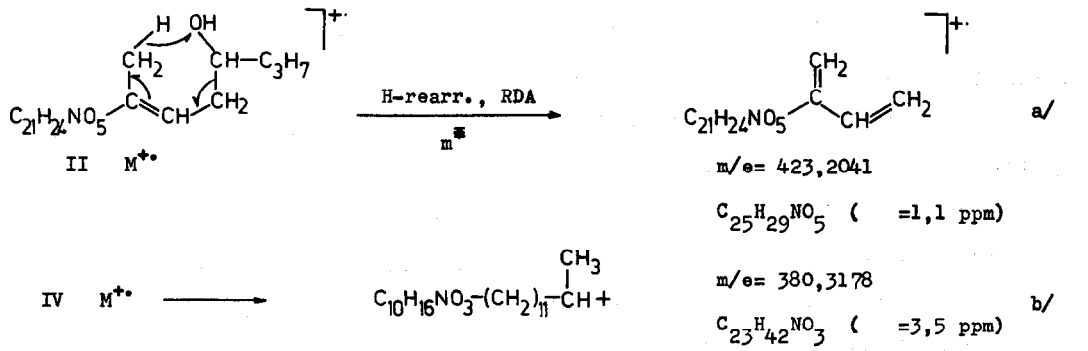


SCHEME 2 *

In the mass spectrum of deuterated IV each fragment ion of Scheme 2 is of the same deuterium content as the molecular ion. The arrangement of $-C_3H_7$ was concluded from NMR spectra (see Table 1 column 1) as being iso-propyl.

4. Structure of the polyenic system: The polyenic system consists of six double bonds as shown by the uptake of 6 H_2 upon Pd-hydrogenation (cf. MS: $M^{+\bullet}$ II and $M^{+\bullet}$ III). These must have all-trans configuration, as no cis-trans isomerization was achieved by heating compound I with acetic acid: acetone= 1:1 /8/. One of the olefinic bonds is methyl-substituted as shown by the NMR spectra (see Table 1 column 3). The position of this branching within the polyenic system can be postulated as follows:

a) The mass spectrum of II shows an abundant ion at m/e= 423, the formation of which can be rationalized by involving H-rearrangement from the branching methyl group, as depicted in Scheme 3/a, triggered by the retro-Diels-Alder reaction /9/ of the last olefinic bond. In accordance with this interpretation no analogous process is observed in the spectra of III and IV.



SCHEME 3 *

* See footnote of Scheme 1.

b) Branched aliphatic chains cleave more probably at the branching upon electron impact /10/. Ion m/e= 380 in the spectrum of IV could be attributed to such a process (shown in Scheme 3/b), as it didnot contain deuterium in MS of labelled IV, while all the other ions formed by the fragmentation of the side chain did. This fact may indicate a primary cleavage, and thereby the position of the branching.

5. It derives from comparison of II with its known structural components, that a $-CH_2-$ group must be inserted in between the polyenic system and the $-CH(OH)-C_3H_7$ end of the side chain to obtain the measured elemental composition of II.

To conclude, structure II represents oleficin-aglycone-methyl-ester and consequently structure I can be assigned to oleficin. Relying upon its published data /5/, α-lipomycin seems to have structure V.

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