MELINACIDINS II, III AND IV
STRUCTURAL STUDIES

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(Received for publication March 22, 1977)

The structures of melinacidins II, III and IV were determined by physicochemical methods. Melinacidin IV is considered to be identical to 11α,11α'-dihydroxychaetocin while melinacidins II and III are isomeric to chaetocin and verticillins A and B.

Melinacidins II, III and IV are antibacterial agents produced by *Acrostalagmus cinnabarinus* var. *melinacidinus*. Melinacidins inhibit a variety of Gram-positive bacteria *in vitro* but were found to be toxic and ineffective in the treatment of bacterial infections *in vivo*. The isolation and characterization of melinacidins have been described earlier[1,2]. The present communication discusses the structure of these antibiotics.

Analytical data and molecular weight determinations (vapor pressure osmometry in chloroform) supported the tentative molecular formulas of $C_{34}H_{34}N_6S_4O_6$, $C_{32}H_{30}N_6S_4O_8$ and $C_{30}H_{30}N_6S_4O_8$ proposed for melinacidins II, III and IV, respectively[2]. However, all three melinacidins have tendency to form solvates with several solvents used for crystallization[2]. Because of this and the spectral evidence discussed below we consider the molecular formulas of $C_{30}H_{28}N_6S_4O_6$, $C_{30}H_{28}N_6S_4O_7$ and $C_{30}H_{28}N_6S_4O_8$ as the correct formulas for melinacidins II, III and IV respectively.

The physical properties of melinacidins II, III and IV (Table 1) indicate close structural relationship among these antibiotics. The infrared spectra of melinacidins are almost identical and show absorption at 3480~3400 cm^{-1} due to NH or OH stretching vibrations. The most characteristic absorption in the spectra is the absorption due to stretching vibration of amide carbonyl groups which appears at *ca* 1685~1665 cm^{-1}. Another characteristic absorption present in the spectra of all melinacidins is the weak absorptions at 1605~1595 cm^{-1} which is tentatively assigned to the presence of an aromatic system in the melinacidin molecules. The infrared spectra of melinacidins and in particular the amide carbonyl bands combined with the positive COTTON effect at 236~237 nm indicate the presence of a dioxopiperazine moiety[3,4,5] in melinacidins II, III and IV. Furthermore,

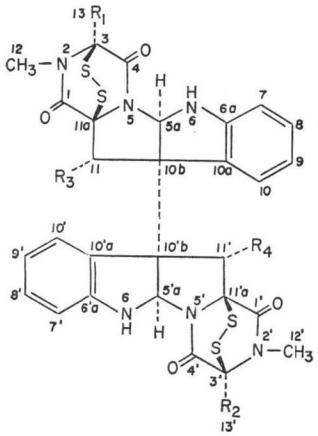
Table 1. Physical properties of melinacidins

	Melinacidin II	Melinacidin III	Melinacidin IV
$[\alpha]_D^{25}$ ($CHCl_3$)	+726° (*c* 0.5)	+776° (*c* 0.5)	+718° (*c* 0.5)
UV (Methanol) λ_{max}, nm (a)	241 (sh) (21) 300 (7.3)	240 (sh) (21) 300 (7.5)	242 (sh) (15) 301 (6.0)
CD (Dioxane) λ_{max}, nm ([Θ])	236 (366,000) 272 (−21,000) 307 (80,300) 375 (−2,050)	237 (383,000) 271 (−21,600) 306.5 (87,600) 375 (−2,250)	237.5 (317,000) 272 (−15,200) 308 (63,000) 374 (−2,540)
IR (Nujol) (cm^{-1})	3450 (sh), 3410, 1685~1670, 1605, 1595 (sh)	3480 (sh), 3405, 1685~1665, 1605, 1595 (sh)	3500 (sh), 3385, 1685~1665, 1605, 1595 (sh)

the COTTON effects at 272, 307 and 375 nm are assigned to a disulfide chromophore[8,6] the presence of which is supported by the mass spectra (*m/e*, 64 due to loss of S_2).[7] These data suggest that melinacidins II, III and IV belong to the group of "3,6-epidithiadiketopiperazine" antibiotics, and most specifically are related to chaetocins[8,9] and verticillins[10,11] the structure of which is shown in Fig. 1.

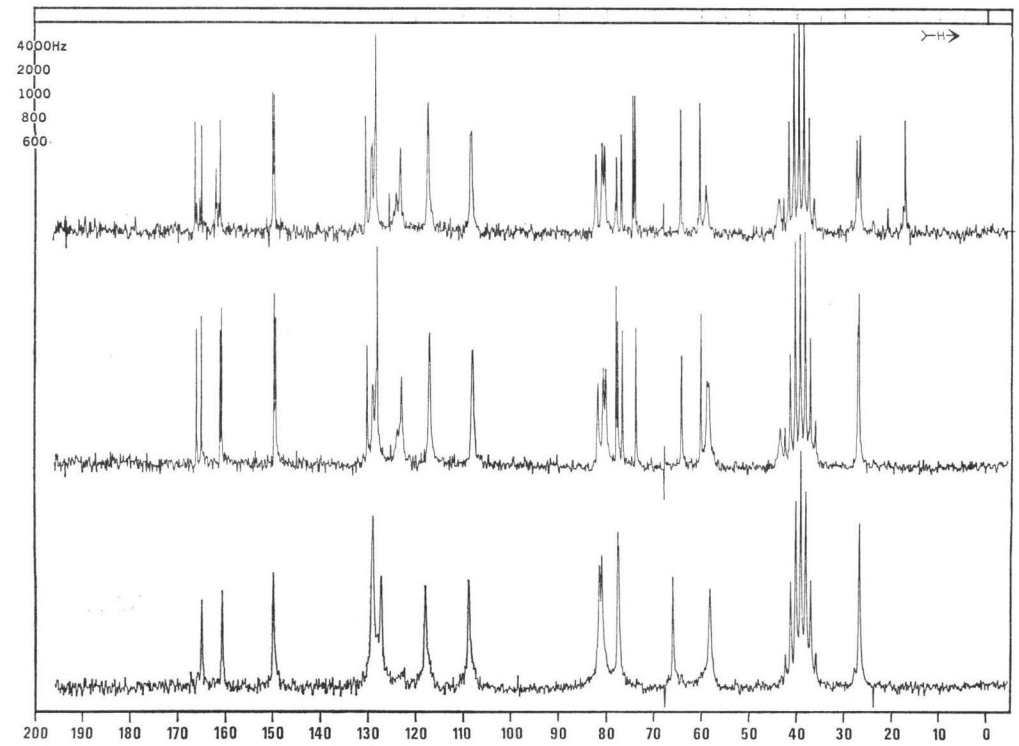
HAUSER, LOOSLI and NIKLAUS[9] reported the isolation and structure of 11α,11α′-dihydroxychaetocin (**3**) and noted the close relationship between **3** and melinacidin IV. A direct comparison of

Fig. 1



		R_1	R_2	R_3	R_4
1	Melinacidin II	$-CH_3$	$-CH_2OH$	-H	-OH
	or	$-CH_3$	$-CH_2OH$	-OH	-H
2	Melinacidin III	$-CH_2OH$	$-CH_2OH$	-OH	-H
3	Melinacidin IV; 11α,11α′-dihydroxy-chaetocin	$-CH_2OH$	$-CH_2OH$	-OH	-OH
4	Chaetocin	$-CH_2OH$	$-CH_2OH$	-H	-H
5	Verticillin A	$-CH_3$	$-CH_3$	-OH	-OH
6	Verticillin B	$-CH_3$	$-CH_2OH$	-OH	-OH

Fig. 2. ^{13}C-NMR Spectra of melinacidin II (upper), melinacidin III (middle) and melinacidin IV (lower).



thin-layer and paper-chromatographic behavior and IR and UV spectra of melinacidin IV and 11α,11α'dihydroxychaetocin* showed that these compounds have very similar, if not identical, structures. The pmr spectra of both melinacidin IV and 11α,11α'-dihydroxychaetocin[9] are identical and characterized by absorption at δ, 2.98 (s, $-NCH_3$, 6H), 4.10 (s, $-CH_2OH$, 4H), 4.85 ($-\underset{|}{C}HOH$, 2H), 5.45 (s, $-NCHN-$, 2H) and aromatic hydrogen absorptions at δ 6.45~7.7 (8H). The cmr spectrum of melinacidin IV (Fig. 2) is identical to that reported for 11α,11α'-dihydroxychaetocin[9]. This is also shown in Table 2 where the cmr chemical shifts of melinacidin IV, determined in the present study, and those reported for 11α,11α'-dihydroxychaetocin[9] are presented. Furthermore the specific rotations and circular dichroism (CD) data of melinacidin IV (Table 1) and 11α,11α'-dihydroxychaetocin[9] are very similar. We conclude therefore that melinacidin IV and 11α,11α'-dihydroxychaetocin are identical and their structure is represented by **3**.

As mentioned earlier, the molecular formulas of $C_{30}H_{28}N_6S_4O_6$ and $C_{30}H_{28}N_6S_4O_7$ are considered for melinacidins II and III instead of the reported[2] $C_{34}H_{34}N_6S_4O_5$ and $C_{32}H_{30}N_6S_4O_8$ respectively. This conclusion is based on cmr and pmr spectral evidence obtained on exhaustively dried melinacidins II and III.

Noise decoupled and off-resonance decoupled cmr spectra (Fig. 2 and Table 2) show the presence of 30 carbons and 24 carbon-linked hydrogens in melinacidin II. The cmr spectrum (Table 2) indicates the presence of four amide carbonyls (singlets at δ 166.6, 165.2, 162.2 and 161.3), one $-\underset{|}{C}HO-$ (doublet at δ 82.6) and one $-CH_2O-$ (triplet at δ 59.5) which account for the six oxygens present in melinacidin II. Pmr spectra indicated the presence of four exchangeable hydrogens. Two of these protons are parts of a $-CH_2OH$ (δ 4.08) and a $-\underset{|}{C}HOH$ (δ 4.23), the remaining two being part of the two $-NH\cdot CHN-$ (δ 5.25) groupings of melinacidin II. In addition the pmr spectrum shows the presence of a $C-CH_3$ (s, δ 1.91), two $N-CH_3$ (2s, δ 2.94 and 3.04), an isolated $-CH_2-$ (δ 2.85~3.1) and aromatic hydrogens [δ 6.2~7.4 (8H)] in the melinacidin II molecule. These assignments are in complete agreement with cmr data (Table 2).

Similarly, cmr spectra show the presence of 30 carbons and 23 carbon-linked hydrogens in melinacidin III. The cmr spectrum of melinacidin III also indicates the presence of four amide carbonyls (singlets at δ 166.6, 165.6, 161.6, 161.3), one $-\underset{|}{C}HO-$ (doublet at δ 82.4) and two $-CH_2O-$ groups (triplets at δ 59.3 and 59.5) which account for the seven oxygens of melinacidin III. Pmr spectra of melinacidin III show the presence of two $-CH_2OH$ (δ 4.05), one $-\underset{|}{C}HOH$ (δ 4.25) and two $-NH-\underset{|}{C}HN$ (δ 5.45) groups accounting for five exchangeable hydrogens. The spectrum shows two $-CH_2OH$ groups and no $C-CH_3$ in melinacidin III instead of one $C-CH_3$ and one CH_2OH in melinacidin II. Otherwise the pmr spectrum of melinacidin III is identical to that of melinacidin II [$2N-CH_3$ (2s, δ 3.02; 3.05); $-CH_2-$ (δ 2.80~3.1) and aromatic hydrogens at δ 6.2~7.45].

Spectral (IR, UV, pmr, cmr) data discussed thus far indicate that all three melinacidins have closely related structures. It is concluded therefore that melinacidins II and III have the basic dimeric structure (Fig. 1) postulated for melinacidin IV and that they differ from each other in the nature of substituents at C_{13},C_{13}' and C_{11},C_{11}' of **1**, **2** and **3** respectively. Specifically, melinacidin IV (**3**), as discussed earlier, contains two $-CH_2OH$ (C_{13}, C_{13}') and two $>CHOH$ (C_{11},C_{11}') groups. Melinacidin III (**2**) contains two $-CH_2OH$ (C_{13},C_{13}') and one $>CHOH$ (C_{11}) while melinacidin II (**1**) contains $-CH_2OH$

* The senior author expresses his gratitudes to Dr. DANIEL HAUSER for the sample of 11α,11α'-dihydroxychaetocin.

Table 2. ^{13}C-NMR spectra of melinacidins II, III, IV and 11α,11′α-dihydroxychaetocin. Chemical shift (δ)[a]

Melinacidins[b,c]			11α,11′α-Dihydroxy-chaetocin (3)[c,e]	Assignment[d]
II (1)	III (2)	IV (3)[e]		
165.2 (s) 166.6 (s)	165.6 (s) 166.6 (s)	165.7 (s)	165.8 (s)	C-4; C-4′
162.2 (s) 161.3 (s)	161.6 (s) 161.3 (s)	161.4 (s)	161.5 (s)	C-1; C-1′
149.9 (s) 150.2 (s)	149.9 (s) 150.2 (s)	150.5 (s)	150.5 (s)	C-6a; C-6′a
128.9 (d) 129.7 (d)	129.0 (d) 129.8 (d)	129.9 (d)	129.9 (d)	C-10; C-10′
129.9 (s) 130.9 (s)	128.8 (s) 130.9 (s)	129.9 (s)	129.9 (s)	C-10a; C-10a′
124.5 (d) 123.7 (d)	124.6 (d) 123.7 (d)	128.2 (d)	128.2 (d)	C-8; C-8′
117.9 (2d)	117.9 (2d)	118.9 (d)	119.1 (d)	C-9; C-9′
108.8 (d) 109.0 (d)	108.9 (2d)	109.9 (d)	110.0 (d)	C-7; C-7′
82.6 (d) 44.5 (t)	82.4 (d) 44.4 (t)	82.3 (d)	82.4 (d)	C-11; C-11′
80.8 (d) 81.3 (d)	80.8 (d) 81.3 (d)	81.8 (d)	81.9 (d)	C-5a; C-5′a
74.9 (s) 78.3 (s)	78.6 (s) 78.3 (s)	78.4 (s)[f]	78.4 (s)[f]	C-3; C-3′
74.5 (s) 77.3 (s)	74.5 (s) 77.3 (s)	78.0 (s)[f]	78.2 (s)[f]	C-11a; C-11a′
60.8 (s) 64.9 (s)	60.8 (s) 65.0 (s)	66.9 (s)	66.9 (s)	C-10b; C-10b′
17.34 (q) 59.5 (t)	60.8 (t) 59.5 (t)	59.1 (t)	59.1 (t)	C-13; C-13′
26.9 (q) 28.2 (q)	28.0 (q) 28.1 (q)	28.0 (q)	28.0 (q)	C-12; C-12′

[a] Relative to tetramethylsilane using d_6-dimethylsulfoxide as an internal standard.
[b] d_6-Dimethylsulfoxide was used as solvent.
[c] Multiplicities in the off-resonance decoupled spectra; s=singlet, d=doublet; t=triplet; q=quartet.
[d] See structures in Fig. 1.
[e] Each carbon signal is due to two corresponding carbons of the monomers in **3** (see text).
[f] Assignments could be reversed.

and $-CH_3$ groups at C_{13},C_{13}′ and one >CHOH (C_{11} or C_{11}′). The exact position of the secondary hydroxyl group in melinacidin II (see Fig. 1) cannot be deduced from the available spectral and other physicochemical data.

It should be noted that melinacidin IV has a symmetrical dimeric structure while melinacidins II and III have a dimeric structure consisting of two slightly differing monomers. This lack of symmetry is responsible for the fact that the cmr chemical shifts of the corresponding carbons in the monomers of melinacidins II and III (Fig. 2; Table 2) differ slightly in contrast to the identical chemical shifts of the corresponding carbons of the monomers of melinacidin IV, or 11α,11α′-dihydroxychaetocin[9]. In addition, the pmr signal given by the two N–CH_3 groups of melinacidins, chaetocin and verticillins appear as a singlet (6H) in the spectra of the symmetric chaetocin (**4**), 11α,11α′-dihydroxychaetocin (melinacidin IV) (**3**) and verticillin A (**5**)[8,9,11]. On the other hand, the pmr spectra of the asymmetric

melinacidin II, melinacidin III and verticillin B (**6**) show the presence of two singlets (3H each) for the $-NCH_3$ groups.

As shown in Fig. 1, melinacidin II is isomeric to verticillin A and chaetocin and melinacidin III is isomeric to verticillin B. The CR data (Table 1) of melinacidins are very similar to those reported for verticillins A and B (II) and 11α,11α'-dihydroxychaetocin[9]. We conclude, therefore, that the stereochemistry at the asymmetric centers (C_3,C_3'; C_{11a},C_{11a}') in the dioxopiperazine ring of melinacidins is identical (S-configuration) to the stereochemistry of chaetocin and 11α,11α'-dihydroxychaetocin determined by HAUSER and his coworkers[8,9]. Furthermore, on the basis of biosynthetic considerations, we propose that the 11-hydroxyl group of melinacidins II and III (like those of melinacidin IV) has the α-configuration.

Experimental

Preparation of Melinacidin

Fermentation as well as isolation procedures which yielded crystalline melinacidin have been reported by ARGOUDELIS and REUSSER[1]. The crystalline mixture obtained by these methods was used as the starting material for isolation of melinacidins II, III and IV.

Isolation of Melinacidins II, III and IV

The procedures described by ARGOUDELIS[2] were used for the separation of melinacidins II, III and IV. The colorless crystalline antibiotics were dried in high vacuum for extended period of time (15~20 days) before use for spectroscopic studies.

Paper and Thin-Layer Chromatographic Procedures

Melinacidins II, III and IV were differentiated from each other and from other related antibiotics by paper chromatography using benzene - methanol - water (1: 1: 2) as the solvent system. Antibiotics were detected by bioautography on *B. subtilis*-seeded agar.

Thin-layer chromatograms were run on silica gel G using toluene - ethyl acetate mixtures (50: 50 or 60: 40, v/v) or methylene chloride - ethyl acetate (70: 30, v/v) as solvent systems. Antibiotics were detected either by bioautography (see above) or by spraying with periodate-permanganate spray reagent.

Spectroscopic Methods

Proton magnetic resonance spectra were recorded on a Varian XL-100-15 spectrometer operating at 100 MHz. Solutions (*ca* 0.4 ml, *ca* 0.25 M) of the compounds in d_6-dimethylsulfoxide were used.

Carbon magnetic resonance spectra were recorded on a Varian CFT-20 spectrometer. Pmr and cmr chemical shifts are reported as ppm relative to tetramethylsilane as internal standard.

Infrared spectra were obtained in mineral oil suspension using a Digilab Model 14D spectrometer.

Acknowledgements

The authors wish to express their appreciation to members of the Physical and Analytical Chemistry Department for spectra and analytical data. The technical assistance of Mr. K. J. GEIPEL is highly appreciated.

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