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Nonactin, Monactin, Dinactin, Trinactin, and Tetranactin. A Raman Spectroscopic Study

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Synopsis

Raman spectra are reported for crystalline nonactin, monactin, dinactin, trinactin, and tetranactin and their solutions in CCl_4, $CHCl_3$, CH_3OH, and 4:1 (v/v) CH_3OH:$CHCl_3$. The macrotetrolide nactins selectively bind a wide variety of cations, and are important model compounds for the study of ion complexation. The conformations of nonactin, monactin, and dinactin in solution are similar. Their conformations are found to be sufficiently open to permit the ester carbonyl groups to form hydrogen bonds with CH_3OH; this gives rise to characteristic changes in the vibration frequencies associated with the ester groups. Nonactin, which is the least soluble of the nactins in CH_3OH, is also the least effective at forming hydrogen bonds with CH_3OH. The greater ability of the higher nactins to form hydrogen bonds with CH_3OH may be due to the increased inductive effect of ethyl over methyl side chains, which may increase the dipole moment of the ester carbonyl groups. Spectra of crystalline nonactin, monactin, and tetranactin are fairly similar, while the spectra of dinactin and trinactin comprise a second, distinct family. This is consistent with X-ray crystallographic studies, which show that nonactin and tetranactin form monoclinic crystals, while trinactin is triclinic.

INTRODUCTION

The nactins (nonactin, monactin, dinactin, trinactin, and tetranactin) are a family of macrocyclic antibiotics isolated on the basis of their antimicrobial[1] and miticidal[2] activity. Figure 1 shows the primary structure of the nactins; composed of alternating tetrahydrofuran rings and ester linkages, they differ from each other in that successive methyl groups (indicated by the asterisk in the figure) are replaced with ethyl groups. The crystal structures of two nactins (nonactin[3] and tetranactin[4]) and several of their cation complexes[5] are known.

The nactins apparently owe their biological activity to their ability to form complexes with simple cations.[6] Given a suitable anion, the cation complexes are soluble in nonpolar solvents such as dichloromethane;[7] in fact, measuring the extraction of alkali picrates into an organic solvent is a standard technique for determining nactin concentration.[8] Cation

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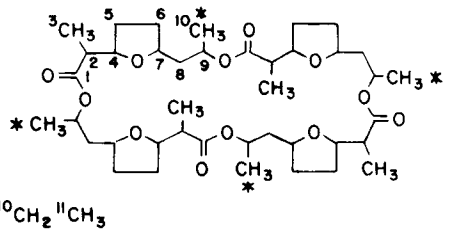

Fig. 1. Primary structure of the macrotetrolide nonactin, indicating the numbering of the carbon atoms. One, two, three and four of the indicated methyl groups are replaced with ethyl groups in the homologs monactin, dinactin, trinactin, and tetranactin, respectively.

complexation is selective; and the equilibrium binding constants of the nactins for different alkali ions differ by several orders of magnitude.[7,9] This selectivity has been studied by a variety of techniques, including measurement of the extraction of cations into bulk phase dichloromethane[9] and observation of the cation concentration dependence of the proton magnetic resonance spectrum.[10,11]

While it is generally agreed that the cation–nactin interaction is primarily electrostatic, several models for the ionic selectivity of the nactins have been proposed. One model emphasizes the geometric arrangement of the oxygen atoms of the nactin and the energy required to distort the nactin to fit around cations of different sizes;[12] a second model ascribes the selectivity entirely to the difference between the cation–solvent and cation–nactin electrostatic interaction energies,[13] while a third model includes both electrostatic and steric factors.[14]

We have obtained Raman spectra of the five nactins and their cation complexes. This paper reports the spectra of the uncomplexed nactins in the solid state and in solution. Comparison is made with the known crystal structures of nonactin and tetranactin. The effects of solvent polarity and hydrogen bonding in nonactin, monactin, and dinactin are studied. This information is of specific interest in understanding the mechanisms for the selectivity of these compounds, which are able to complex with a particularly wide variety of simple cations. A more detailed understanding of ionic selectivity in the nactins may provide a model for understanding ionic selectivity in other, more complex systems. Some preliminary results on nonactin complexation have been described elsewhere.[15,16]

EXPERIMENTAL

Raman spectra were obtained using a SPEX Ramalog 4 double-grating monochromator with cryogenically cooled RCA GaAs photomultiplier tube, and a Spectra-Physics model 164-03 argon ion laser. Laser plasma lines were eliminated with a Claasen filter. Samples were held in Kimax glass capillaries (1.7 mm inner diameter) mounted perpendic-

ular to the scattering plane. The polarization vector of the incident laser light was perpendicular to the scattering plane; a polarization scrambler was permanently mounted in front of the first monochromator slit. Most measurements were performed using the 5145- and 4880-Å laser lines; in some cases, sample fluorescence was reduced by using the 4579-Å laser line.

The nonactin was the gift of Dr. B. Stearns of the Squibb Institute for Medical Research; the monactin and dinactin were gifts of Drs. H. Bickel and K. Scheibli of Ciba-Geigy, Ltd. An initial sample of trinactin was generously provided by Prof. G. Eisenman of U.C.L.A.; samples of tetranactin and trinactin were gifts of Drs. Y. Nawata and K. Ando of the Chugai Pharmaceutical Co., Ltd. All were used without further purification. The solvents used were spectroscopic grade. Antibiotic solutions (0.03 *M*) were prepared directly in the capillaries and then centrifuged.

Spectral line assignments were made by comparison with published normal mode calculations and group frequency analyses of model com-

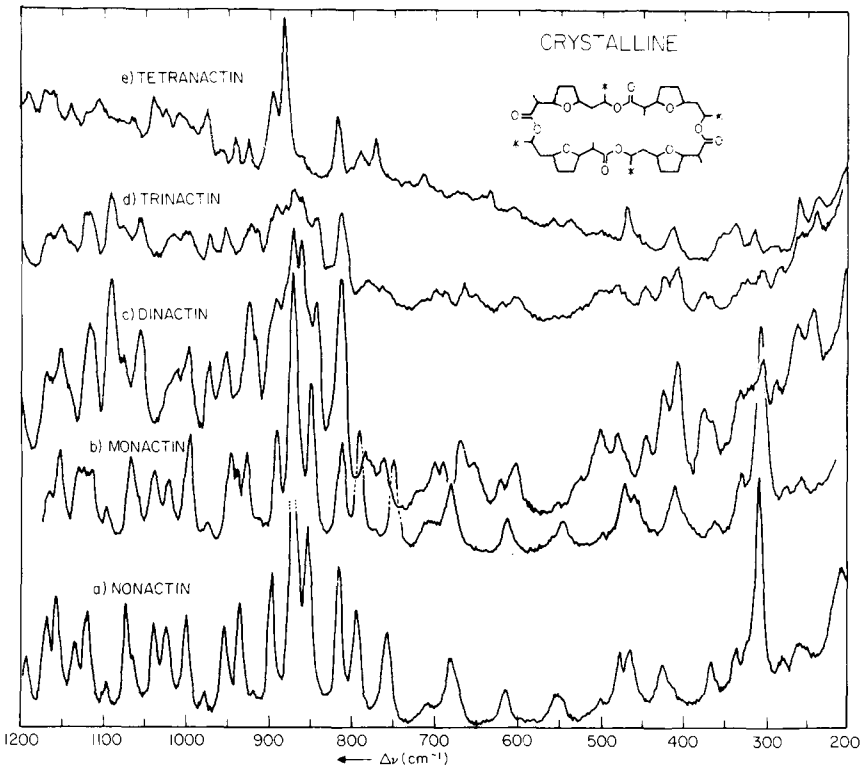

Fig. 2. Raman spectra of crystalline nonactin, monactin, dinactin, trinactin, and tetranactin in the region 200–1200 cm^{-1}. Resolution is 2.5 cm^{-1} for nonactin, monactin, and dinactin; 5 cm^{-1} for trinactin and tetranactin. 20–100 mW laser power (4579, 5145 Å), and a scanning rate of 30 cm^{-1}/min were used.

TABLE I

Raman Spectra of the Crystalline Nactins in the Range 200–3100 cm^{-1}

Nonactin	Monactin	Dinactin	Trinactin	Tetranactin	Assignments
	156	156	154	156	
		171	173		
	197	195	~200	198	O ‖ —C—O— torsion
		242	241	238	
255	258	261	260	261	
276	277	287	284	294	
305	304	305	305		
318	316	315	(?)	314	In-plane skeletal bend, including ester
332	331	331	330	331	
363	364	364	367	368	
		375	376		
	410	407	408	411	O—C—C skeletal bend
422		424	425		
		446	447		
461	458			465	
475	470	481	480		Tetrahydrofuran ring def?
498		503	502		
		530		535	CH_2—O—C bend?
546	546	552		557	
610	613	603	604	605	Ester (def); C=O out-of-plane bend
		620	621	632	
		653	653		
		667	666	664	
672	680	687	688	672	
703	707	698	700	711	Ester C=O in-plane bending
		719	719		
753	751	762	764	761	CH_2 rock-twist
				768	
789	792	783	783	788	
810	812	812	813	818	Methyl side chain C—C stretch
847	847	841	844		Ester C—O—C
		861	861	862	Sym stretch
866	870	870	872		Tetrahydrofuran C—O—C
		877?		881	Sym stretch
891	891	891	893	895	CH_3 in-plane rock +
914	915	915	914		C—C stretch?
930	926	923	923	925	
	940			941	Ester: skeletal def + methyl rock (?)
948	946	951	953		
				959	
975	974	972	973	975	CH_2 twist-rock
994	994	997	1000	1002	
		1010	1017	1010	CH_3 side-chain
1020	1020	1020		1024	
1035	1038	1055	1055	1038	CH_3 def + C—C stretch
1068	1067	1074	1076	1063	C—O—C asym stretch (tetrahydrofuran)
1093	1095	1090	1091		Ester O—C—C asym stretch
				1104	
1116	1113	1116	1119	1118	
	1122				Asym CH_3 rock (C^{10}, C^{11})
1131	1128			1138	
1158	1153	1151	1151	1158	Asym CH_3 rock (C^3)
1163	1165	1166	1166	1168	
1189	1195			1189	C—O—C asym stretch (ester)
1202	1203	1203	1202	1197	
				1214	
	1232	1227	1226	1228	C^{10} (ethyl) CH_2 twist-rock (?)
1245	1248	1237	1241		CH_2 twist-rock
1258	1263	1264		1260	
		1275	1275	1271	
				1286	

(*continued*)

TABLE I (*continued*)

Nonactin	Monactin	Dinactin	Trinactin	Tetranactin	Assignments
1294	1292	1293	1294		
1314	1315			1305	
		1326	1326	1323	CH_2 wag, C—H def
1332	1333				
1341	1341	1341	1340	1343	
1350					
1357	1355	1358	1357	1358	
1364	1367		1365	1366	
1380	1380	1382	1383		CH_3 sym bend
1396	1396				
1420	1422	1417	1417	1428	C^{10} CH_3 asym def
1440	1440				CH_2 bending, CH_3
1451	1451	1450	1449	1450	Asym def
1467	1465	1462	1462	1471	
1723	1726	1728	1730	1728	Ester C=O stretch
1732					
2759	2740	2735		—	Overtone vibration
2778	2780				
2855				2853	CH_2sym stretch
	2863	(2866)			
				2874	CH_3 sym stretch
2891	2888	2881	2882	2890	
2906					CH stretch (C^4, C^7)
2921	2920	2911	2913	2920	CH stretch
		2927	2926	2928	CH_2 asym stretch (C^5, C^6)
2942	2946	2939	2942	2942	CH_2 asym stretch (C^8?)
	(2958)	2959	2954	2956	CH_2 asym stretch (C^{10}?)
2966					
2979	2972	2974	2976	2968	CH_2 asym stretch (C^3?)
2986	2987			2982	
2999	2994			3006	(C^{16}, C^{11}?)

sym = symmetric; asym = asymmetric; def = deformation; sh = shoulder.

pounds. The use of a homologous series of antibiotics greatly aided the detailed assignment of Raman lines. Dissolving these compounds in different solvents eliminated crystal field splittings, and provided information about solvent effects such as hydrogen bonding.

RESULTS

Although the spectra of the five nactins in CCl_4 solution are rather similar, substantial differences are observed in crystalline nactins, which arise primarily from intermolecular interactions. The spectra of crystalline nonactin, monactin, and tetranactin appear to form one family, while those of crystalline dinactin and trinactin form a second family. Several spectral lines that are singlets in one family are doublets in the other, etc. Raman spectra of the five nactins in the crystalline state and dissolved in CCl_4, $CHCl_3$, CH_3OH, and 4:1 (v/v) CH_3OH:$CHCl_3$ are shown in Figures 2–6. The observed Raman frequencies and their assignments are summarized in Tables I–IV, and are discussed in detail below.

150–800-cm^{-1} Region

This region contains primarily structural deformations and torsional modes. The ester deformation modes have been analyzed by Tadokoro

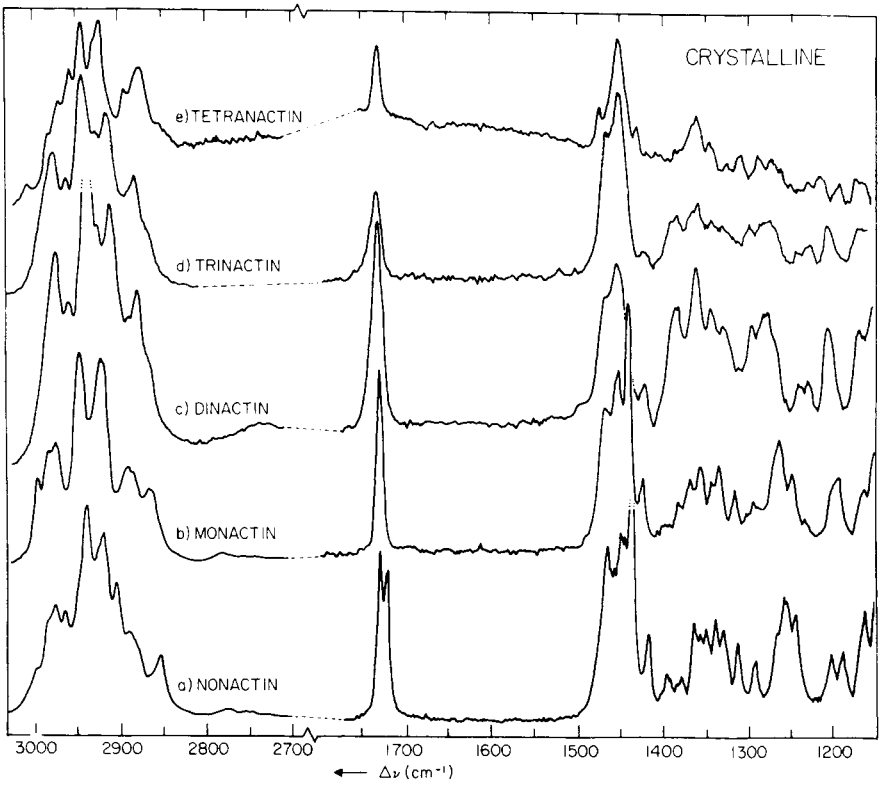

Fig. 3. Raman spectra of crystalline nonactin, monactin, dinactin, trinactin, and tetranactin in the ranges 1150–1800 cm^{-1} and 2700–3050 cm^{-1}. Experimental conditions as in Fig. 2.

et al.[17] who compared normal mode calculations with the far-infrared spectra (<750 cm^{-1}) of a series of homologous aliphatic polyesters, to identify the C=O in-plane bending (690–730 cm^{-1}), the C=O out-of-plane bending (530–590 cm^{-1}), O—C—C bending (430–460 cm^{-1}), CO—O torsion (190–240 cm^{-1}), and CH_2—O torsion (<150 cm^{-1}) modes. In compounds in which sufficient space exists between ester groups, the C—O—C bending mode appears near 520 cm^{-1}. Similar bands are found in the esters of propionic, butyric, and isobutyric acid near 610, 630, 750 cm^{-1}. Many of the nactin lines observed in this region can be assigned in analogy with these model compounds (Table I).

Crystalline nactins display several spectral differences in this region due to differences in crystal structures and in the number of ethyl groups in the four C^{10} positions (Figure 1). Because intense solvent lines prevent comparison with solution spectra over much of this region, these effects are difficult to distinguish. For example, the intense 305-cm^{-1} line of nonactin and monactin is weaker in dinactin and trinactin, and is absent in tetranactin (Figure 2). This line probably represents a skeletal bend, which includes the ester oxygen (Figure 1); it is apparent-

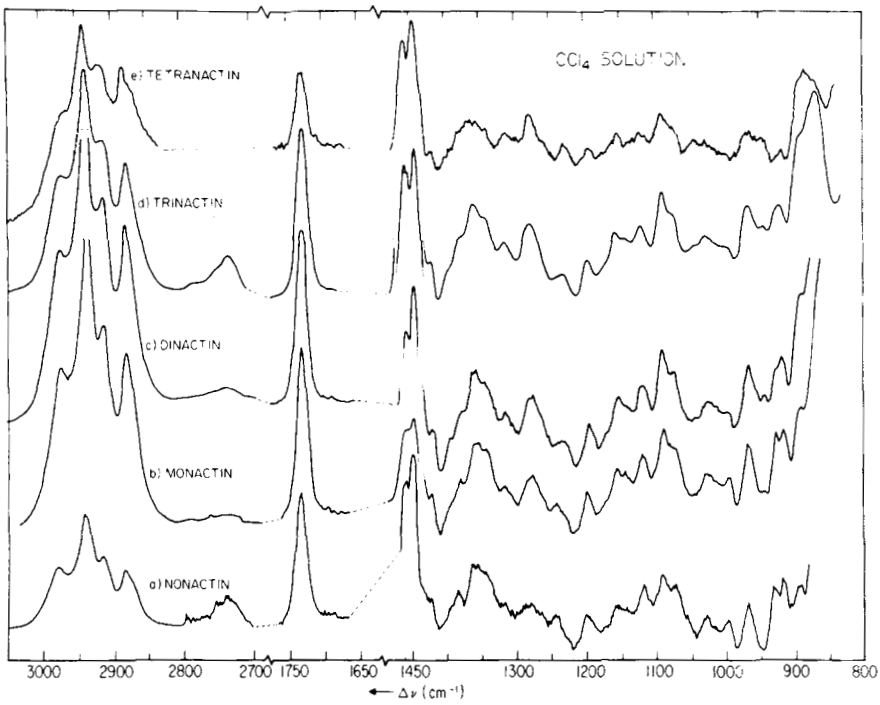

Fig. 4. Raman spectra of CCl_4 solutions of nonactin, monactin, dinactin, trinactin, and tetranactin in the ranges 850–1800 cm^{-1} and 2700–3050 cm^{-1}. The illumination was 100–200 mW at 4880 Å; resolution is 5 cm^{-1}. Scanning speed was 30 cm^{-1}/min (nonactin, trinactin, tetranactin) or 60 cm^{-1}/min (monactin, dinactin).

ly affected by the presence of methyl groups at position C^{10}. Conversely, the peak near 240 cm^{-1} may be associated with ethyl groups at C^{10}. Several bands of nonactin and monactin (e.g., those near 363, 415, 612, 675, and 705 cm^{-1}) are each doublets in dinactin and trinactin but not tetranactin; they may arise from intermolecular crystalline forces.

In contrast with crystalline samples, $CHCl_3$ solutions of nonactin, monactin, and dinactin are similar over the limited low-frequency region (430–630 cm^{-1}) in which they can be observed. In particular, the doublets near 460, 472 cm^{-1} in crystalline nonactin and monactin, and near 480, 502 cm^{-1} in crystalline dinactin and trinactin, appear near 500, 520 cm^{-1} in $CHCl_3$ solution (Table III). Comparison with spectra of model compounds suggests that this doublet may involve C—O—C bending of the tetrahydrofuran ring or the ester group.

800–1000-cm^{-1} Region

Several differences between crystalline tetranactin and nonactin (or monactin) appear in the 800–1000-cm^{-1} region, although spectra of these compounds are usually similar. A strong peak at 881 cm^{-1} with a shoulder at 862 cm^{-1} is observed only in tetranactin. In crystalline

nonactin and monactin there is a pair of peaks at 847, 870 cm^{-1}, each of which is split in dinactin and trinactin (842, 861, 870, and 877 cm^{-1}). The 800–900-cm^{-1} regions of the crystalline nactins all contain a strong peak near 812 cm^{-1} and a weak peak near 893 cm^{-1}.

By comparison with isoalkanes containing tertiary carbon atoms, we identify the 812-cm^{-1} line as C—CH_3 stretch of the methyl side chains;

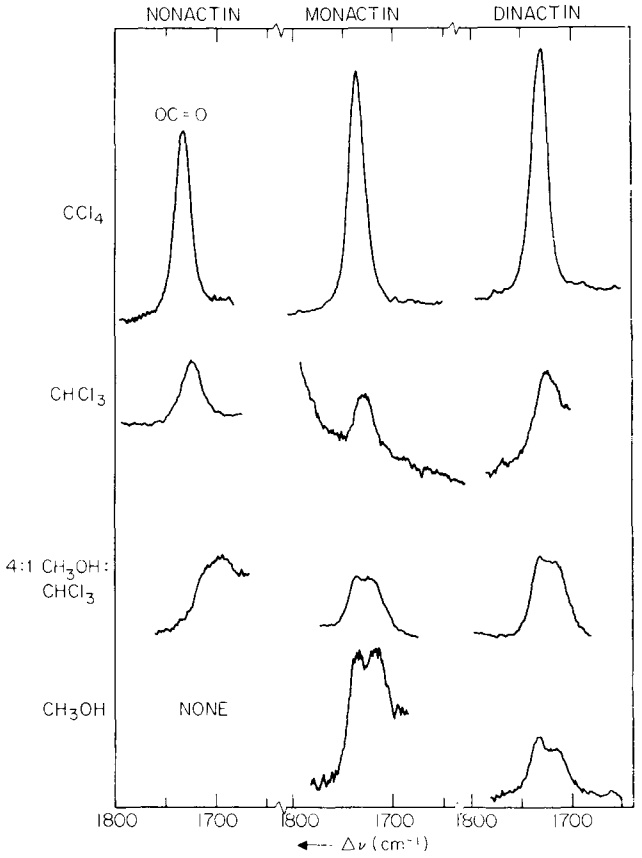

Fig. 5. Raman spectra of the 1700-cm^{-1} region of nonactin, monactin, and dinactin in CCl_4, $CHCl_3$, 4:1 CH_3OH:$CHCl_3$, and CH_3OH at a resolution of 5 cm^{-1} showing the effects of solvent polarity and hydrogen bonding. (Nonactin is not sufficiently soluble in methanol to give a good spectrum). Laser power was 100–400 mW; scanning rate was 30 cm^{-1}/min.

methyl side-chain vibrations also appear near 1010, 1030–1050, and 1155 cm^{-1} both in the isoalkanes and in the nactins. The 891-cm^{-1} line of the nactins occurs at the same frequency as the highly localized methyl in-plane rock/C—C stretch mode found by Snyder and Schachtschneider in long-chain hydrocarbons.[18,19] In short linear hydrocarbons, interaction between the methyl groups at the two ends of the molecule splits this line into a doublet. Although the nactins contain several

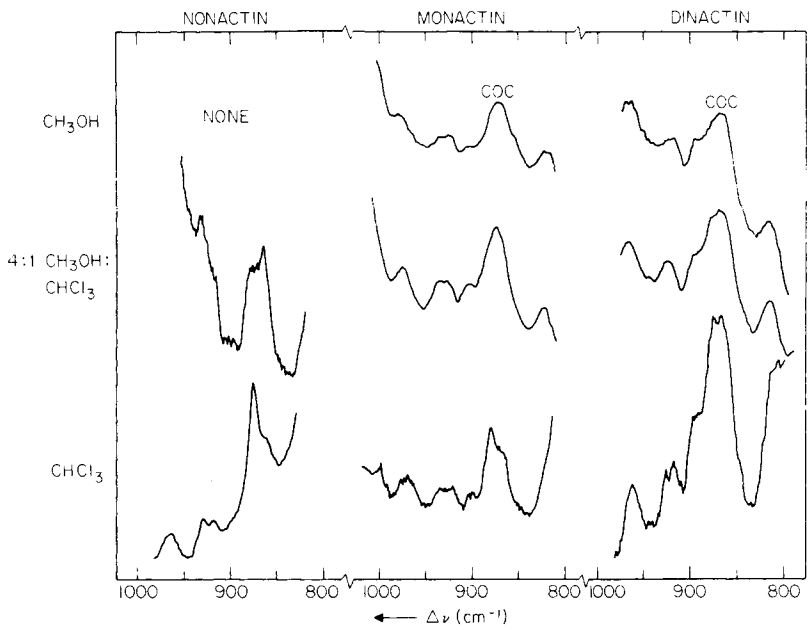

Fig. 6. Raman spectra of nonactin, monactin, and dinactin in $CHCl_3$, 4:1 v/v CH_3OH: $CHCl_3$, and CH_3OH. The relative effect of a hydrogen-bonding solvent on the ester (860–870 cm^{-1}) and tetrahydrofuran (875–880 cm^{-1}) is especially visible in the nonactin and monactin spectra. Laser power was 120–400 mW, scanning rate was 30 cm^{-1}/min; resolution is 5 cm^{-1}.

ethyl and methyl side chains, they are isolated from each other by the rest of the molecule.

The intensity and frequency of the 840–880-cm^{-1} lines suggest that they correspond to the C—O—C symmetric stretch modes of the tetrahydrofuran rings and ester linkages.[20–22] Pitzner[23] has identified the 880-cm^{-1} line of the 1,5-anhydropentitols as corresponding primarily to the symmetric ring stretch. Hydrogen bonding has a noticeable effect on this region (Figure 6). Methanol enhances the intensity of the 860-cm^{-1} peak and shifts it to a higher frequency. Comparison of the spectra of 5% (v/v) solutions of methyl acetate and of 2,5-dimethyl tetrahydrofuran in $CHCl_3$ and in CH_3OH show that CH_3OH increases the frequency of the ester peak by several cm^{-1} (perhaps by hydrogen bond formation) while leaving the tetrahydrofuran peak nearly unchanged (<0.5-cm^{-1} shift). This allows the 840–862-cm^{-1} peaks to be identified with the ester C—O—C symmetric stretch and the 866–881-cm^{-1} peak to be identified with the tetrahydrofuran C—O—C symmetric stretch.

Lines near 920 and 967 cm^{-1} are a general feature of the infrared spectra of *n*-propyl, isopropyl, butyl, and isobutyl esters; Katrizky et al.[24] suggest that these lines involve coupled methyl rock and skeletal stretch modes. Such lines occur with equal intensity in the spectra of all five nactins. As methyl groups are replaced by ethyl groups, the

TABLE II
Raman Spectral lines of CCl_4 Solutions of the Nactins

Nonactin	Monactin	Dinactin	Trinactin	Tetranactin	Assignments
865			864	872	C—O—C sym stretch
				885	(tetrahydrofuran?)
894	891	895	895	895	CH_3 in-plane rock + C—C stretch
917	918	920	920	920	Ester vibration (skeletal def + CH_3 rock?)
930	929	920	927		
		944	945	947	CH_3 rock?
967	967	967	966	967	
997	995	996	1001		(—CH_3 side-chain)
1029	1024	1024	1027	1025	Ester vibration
			1043	1045	CH_2 twist-rock, CH_3 side-chain
1073	1073	1074	1074	1077	C—O—C asym stretch (tetrahydrofuran)
1092	1090	1090	1090	1090	Ester (O—C—C asym stretch)
1119	1120	1120	1122	1119	Ester vibration
	(1144)	1142	1141		Asym —CH_3 rock (?)
1155	1157	1153	1156	1155	
		1174	1173 sh		
1201	1197	1197	1198	1196	Ester (C—O—C asym stretch)
1253	1242	1232	1233	1230	
				1254	CH_2 twist-rock
1281	1277	1279	1280	1280	
1317	1317	1317	1318	1314	
1343	1341	1340	1344	1342	CH_2 wag, C—H def
1362	1355	1359	1361	1363	
1383	1380	1382	1381	1382	CH_3 sym bend
	1396?	1397	1397	1396	
1422	1421	1420	1423	1422	$C^{10}CH_3$ bend (?)
1448	1447	1446	1446	1446	CH_2 bend
1458	1459	1459	1459	1460	CH_3 asym def
1733	1733	1733	1733	1733	Ester C=O stretch
~2739	~2735	~2738	~2740		Overtone frequencies
	~2788		~2782		
2873 sh	2874 sh	2871 sh	2873 sh	2870	CH_2 sym stretch
2885	2882	2883	2884	2883	CH_3 asym stretch
2917	2915	2915	2916	2919	CH stretch
2944	2939	2940	2942	2942	CH_2 asym stretch
2981	2975	2974	2975	2970	CH_3 asym stretch

948-cm^{-1} peak of the crystalline nactins decreases in intensity, and is gradually replaced by a peak at 940 cm^{-1}. Similarly, in CCl_4 solutions at the 930 and 997 cm^{-1} peaks decrease in intensity, to be replaced (in tetranactin) by peaks at 974 and 1002 cm^{-1}.

1000–1200-cm^{-1} Region

The infrared spectra[18,24] of *n*-propyl, isopropyl, butyl, isobutyl, and *s*-butyl esters show characteristic peaks near 1020, 1090, and 1120 cm^{-1}. Colthup et al.[21] suggest that the 1190–1210-cm^{-1} line seen in many esters corresponds to the C—O—C asymmetric stretch, while lines in the 1050–1100-cm^{-1} region correspond to the O—CH_2—C asymmetric stretch vibration. The analysis of the vibrational spectra of the 1,5-anhydropentitols by Pitzner[23] suggests identifications for vibrations of the tetrahydrofuran rings. In particular, the 1,5-anhydropentitols have coupled C—O—C stretch and ring C—C stretch lines near 1020, 1060, 1090, and 1130 cm^{-1}; peaks near these frequencies appear in the nac-

TABLE III
Spectra of $CHCl_3$ Solutions of Nonactin, Monactin, and Dinactin

Nonactin	Monactin	Dinactin	Assignments
500	~500	495	
521	514	519	Tetrahydrofuran ring distortion (?) or
611			Ester COC bend
		814	—CH_3 C—C stretch
		864	Ester C—O—C sym
863	860	869	stretch
878	876	878	Tetrahydrofuran C—O—C sym stretch
	899	896	CH_3 in-plane rock + C—C stretch
919	918	919	
930	931	927	Ester (skeletal def + CH_3 rock
966	966	963	CH_3 rock/CH_2 rock-twist (?)
994	994	994	C—C stretch associated with —CH_3
1034	1031	1028	Ester vibration
1075	1076	1072	Tetrahydrofuran C—O—C asym stretch
1090			
1119	1121	1117	Ester vibration (?)
(1141)		1140	—CH_3 rock
		(1156)	—CH_2 rock,
1363	1363	1358	C—H def
1383	1383	1383	CH_3 sym band
1421	1423	1421	C'^{0} CH_3 asym def
1448	1447	1448	CH_2 bend, CH_3 asym def
1458	1458	1458	
1726	1725	1726	Ester C=O stretch

TABLE IV
Comparison of Spectra of Nonactin, Monactin, and Dinactin in 4:1 (v/v) Methanol; Chloroform and in Pure Methanol

Nonactin 4:1 CH_3OH: $CHCl_3$	Monactin 4:1 CH_3OH: $CHCl_3$	Monactin 4:1 CH_3OH: CH_3OH	Dinactin 4:1 CH_3OH: $CHCl_3$	Dinactin 4:1 CH_3OH: CH_3OH	
	423	424	423	427	O—C—C skeletal bend (?)
	485	477	476	477	Tetrahydrofuran ring def
502			508		
520	517	515		516	
			616		
	615–630	615–625	629		Ester C=O out-of-plane bend
		664		662	
		695		699	Ester def?
	817		817	818	—CH_3 C—C stretch
867	867	866	867	866	Ester C—O—C sym stretch
877	878	877	878	~874	Tetrahydrofuran C—O—C sym stretch
897	898	896	898	898	CH_3 in-plane rock + C—C stretch
922	922	918	925	923	Ester (skeletal def +
933	931	929			methyl rock?)
		944			
	968	969	969	967	CH_3 rock/CH_2 rock-twist (?)
1725 (d)	1718	1715	1718	1715	Ester C=O stretch
	1732	1733	1732	1733	

Note: nonactin is not sufficiently soluble in methanol to give a useful spectrum.
(d) = close doublet.

tins. This region also contains methylene coupled twist and rock modes.[19]

The spectra of crystalline nonactin and monactin are essentially the same in this region, as are those of dinactin and trinactin. Spectra of these two families differ near 1035, 1055, 1090, and 1131 cm^{-1}. A number of additional lines are visible in the spectrum of crystalline tetranactin: 1010, 1104 cm^{-1} and a broad band near 1070–1100 cm^{-1}. In CCl_4 solutions of the series nonactin to tetranactin, the 995-cm^{-1} peak is gradually replaced with a peak at 1040 cm^{-1} (an appropriate frequency for the ethyl C^{10} methylene rock-twist mode.)[19] The 1122, 1128-cm^{-1} peaks of monactin (a singlet at 1131 cm^{-1} in nonactin) do not appear in tetranactin, suggesting that they involve C^{10} asymmetric methyl rock, leaving the 1151–1168-cm^{-1} lines to be assigned to C^3 asymmetric methyl rock.

1200–1500-cm^{-1} Region

A series of close contacts between the carbonyl oxygens and the methyl and ethyl groups of tetranactin are found in the X-ray measurements of Nawata et al.[4] A series of close contacts between methyl and methylene groups appears in similar measurements on nonactin.[3] These interactions give rise to changes and splittings in the methyl and methylene bending modes, which abound in this region. Many of these features are characteristic of a given nactin crystal structure and disappear in CCl_4 solution.

n-paraffins display methylene twist and rock vibrations in the 1200–1300-cm^{-1} region,[19] which makes this region a useful one for the spectroscopic study of the effects of replacement of methyl by ethyl groups (position C^{10}, Figure 1), and the effects of different crystalline packings of nactin molecules. For example, the prominent doublet near 1245, 1260-cm^{-1} in crystalline nonactin and monactin may be contrasted with the prominent 1275, 1293-cm^{-1} doublet of dinactin and trinactin (Figure 3). Tetranactin displays a broad triplet near 1260–1286 cm^{-1}, one of the few spectral indications that its crystal structure differs slightly from that of nonactin and monactin. In CCl_4 solution, where interactions between nactin molecules are minimal (Figure 4), all nactins display a broad doublet near 1230–1242 cm^{-1} (1253 cm^{-1} in nonactin) and 1277–1281 cm^{-1}. The ~1227, 1237-cm^{-1} doublet of crystalline dinactin and trinactin may arise from ethyl CH_2 vibrations, especially since small peaks (not present in nonactin), which appear near 1232 cm^{-1} in monactin and 1228 cm^{-1} in tetranactin, do not appear in CCl_4 solution. Crystalline tetranactin lacks the 1293-cm^{-1} peak of the other nactins; this peak may be shifted to 1286 cm^{-1} by intermolecular forces in the crystal. The methyl and methylene vibrations of the 1300–1400-cm^{-1} region are also sensitive indicators of crystal structure. For example, the 1314-cm^{-1} peak of nonactin and monactin (1306 cm^{-1} in tetranactin)

does not appear in spectra of crystalline dinactin and trinactin (Figure 3); however, it appears near 1315 cm^{-1} in CCl_4 solutions of all five nactins (Figure 4).

In linear paraffins[19] and primary esters[24], the 1360–1380-cm^{-1} region contains methyl symmetric bending modes; in o-*sec* butyl esters these modes appear near 1385 cm^{-1}. These peaks are sensitive to crystalline forces in the nactins. In general, there is a decrease in spectral complexity from nonactin and monactin (~1355, 1367, 1380 cm^{-1}) to dinactin (~1358, 1382 cm^{-1}) or tetranactin (1358 cm^{-1}); again, in CCl_4 solution the spectra of all nactins are similar. A comparison of the crystalline nactins (Figure 3) further suggests that vibrations of the methyl side groups appear near 1332 cm^{-1}, while those of the ethyl side groups appear near 1324 cm^{-1}.

The effect of crystal structure is particularly evident for the intense 1440-cm^{-1} peak of nonactin and monactin (methyl asymmetric deformation and methylene bend), which is absent in the other nactins and in solution. The ~1450, 1464 (1471 tetranactin)-cm^{-1} peaks of the crystalline nactins are shifted 4 cm^{-1} downward in CCl_4 solution. The 1420-cm^{-1} peak of nonactin appears to lose intensity as methyl groups at C^{10} are replaced by ethyl groups, suggesting it represents a methyl asymmetric deformation mode.

1600–1800-cm^{-1} Region

The ester carbonyl stretch vibration, which appears near 1715–1735 cm^{-1}, is noticeably affected by solvent polarity. Figure 5 shows the 1700-cm^{-1} region of nonactin, monactin, and dinactin dissolved in CCl_4, $CHCl_3$, CH_3OH, and 4:1 (v/v) CH_3OH:$CHCl_3$. The carbonyl stretch frequency ν_{CO} is near 1733 cm^{-1} in CCl_4, and 7–8 cm^{-1} lower in $CHCl_3$. The decrease in ν_{CO} with increasing solvent polarity is a general phenomenon in esters; the effect is most often attributed directly to the change in the dielectric constant of the medium.[25]

Hydrogen bonding reduces ν_{CO}.[26] In methanol solution, two distinct peaks are seen in the spectra of monactin and dinactin. (Nonactin is not sufficiently soluble in pure methanol to give a good spectrum.) The 1733-cm^{-1} peak is unshifted from its position in CCl_4, while the second peak is down-shifted by ~20 cm^{-1}. The latter presumably arises from hydrogen bonding, which is known to reduce ν_{CO} substantially.[26] Spectra observed in the 4:1 (v/v) CH_3OH:$CHCl_3$ are midway between those observed in pure CH_3OH or $CHCl_3$.

The order–disorder structure of crystalline nonactin[3] is reflected in its Raman spectrum, which displays a doublet at 1724, 1733 cm^{-1}. In contrast, the other crystalline nactins have a single ester carbonyl stretch peak, whose frequency increases monotonically in the series monactin to tetranactin. In nonactin crystals, each plane of molecules is identical, but neighboring layers can be packed in two different ways

without destroying long-range order. These packings provide two different local environments for the ester carbonyl groups, which gives rise to the observed doublet. In the other nactins, only one arrangement of the ester carbonyl groups is observed; this is confirmed by X-ray measurements on tetranactin.[4]

2700–3100-cm^{-1} Region

Although spectra of the CH stretch vibrations (2850–3000 cm^{-1}) of all five nactins are virtually identical in CCl_4 solution (Figure 4), several clear spectral differences are observed in the solid state (Figure 3). For example, crystalline nonactin, monactin, and tetranactin each have three to four peaks in the 2965–3010-cm^{-1} region (methyl asymmetric stretch), while spectra of crystalline dinactin and trinactin contain a singlet near 2975 cm^{-1}. This difference is presumably due to the different crystalline structures of these families of nactins; in solution, all nactins have a singlet near 2975 cm^{-1}. Another example is the 2853–2863-cm^{-1} peak of crystalline nonactin, monactin, and tetranactin, which is at best a vague shoulder near 2870 cm^{-1} in crystalline dinactin, trinactin, and in CCl_4 solution of all nactins.

Studies by Snyder and Schachtshneider[19] indicate that CH stretch frequencies are relatively constant in different paraffins; thus model compounds can be profitably used to help analyze these vibrations in the nactins. For example, the 2971-, 2930-, 2906-, and 2869-cm^{-1} peaks of 2,5-dimethyl tetrahydrofuran can be readily identified as methyl asymmetric stretch, as methylene asymmetric stretch, as tertiary C—H stretch, and as the degenerate methyl and methylene symmetric stretch vibrations, respectively. Comparison of nonactin, monactin, and tetranactin suggests that the 2890-cm^{-1} peak is the *methyl* CH_3 symmetric stretch while the 2874-cm^{-1} peak is the *ethyl* CH_3 symmetric stretch. That the replacement of methyl with ethyl groups should reduce the symmetric CH_3 stretch frequency is suggested by the relative frequencies of this mode in neopentane (2907 cm^{-1}) and *n*-hexane (2875 cm^{-1}).[19] The peaks near 2850–2870 cm^{-1} may be assigned to methylene symmetric stretch.

A comparison of the homologous nactins aids the more detailed analysis of their spectra. For example, the 2958-cm^{-1} peak, barely visible in crystalline monactin, becomes increasingly prominent as ethyl groups replace methyl groups; this band thus probably represents the methylene asymmetric stretch of the ethyl groups. As discussed above, crystal interactions split the virtually degenerate asymmetric stretch modes of the two inequivalent methyl groups in crystalline nonactin, monactin, and tetranactin (2965–3010 cm^{-1}). The replacement of the peaks near 2987, 2994 cm^{-1} in nonactin and monactin by peaks near 2982, 3006 cm^{-1} in tetranactin suggests that these pairs represent the asymmetric methyl stretch vibrations of the methyl and ethyl side groups, respec-

tively (positions C^{10}, C^{11} in Figure 1). By elimination we identify the 2966, 2979-cm^{-1} lines of nonactin (a singlet near 2972 cm^{-1} in other nactins) with the C^3 methyl asymmetric stretch.

In dimethyl tetrahydrofuran, the tertiary hydrogen of the tetrahydrofuran ring (positions C^4, C^7 in Figure 1) have a stretching frequency near 2906–2910 cm^{-1}; this line is visible as a distinct peak in crystalline nonactin and as a weak shoulder on the 2911–2920-cm^{-1} peak of the other nactins. The 2920-cm^{-1} peak probably represents the other tertiary C—H stretches, while the 2927-cm^{-1} peak may correspond to the asymmetric methylene stretch of the tetrahydrofuran ring. The latter frequency is found 3 cm^{-1} lower in the nactins than in the model compound. The intense peak near 2942 cm^{-1} is a common feature of the nactins; its absence in the tetrahydrofuran derivative suggests that this peak is the symmetric methylene stretch vibration of the nactin ester groups.

The lines in the 2700–2750 region of the spectrum are most likely overtone vibrations.[21] Our assignments are summarized in Table I.

CONCLUSIONS

Raman spectroscopy has previously been applied[15,16] to the study of cation complexation and selectivity in nonactin. This paper complements this preliminary work by treating all five macrotetrolide nactins in their uncomplexed forms, both in the crystalline state and in solution. Further work on the cation complexes of the nactins is underway.[27] The Raman spectra of nonactin, monactin, and dinactin in CCl_4, $CHCl_3$, CH_3OH, and 4:1 (v/v) CH_3OH:$CHCl_3$ indicate that the ester carbonyl groups are sensitive to solvent polarity and to hydrogen bonding. This sensitivity suggests that the conformation of the nactins in solution must be relatively open for otherwise steric interactions would block the formation of hydrogen bonds between nactin and methanol. This conclusion is consistent with X-ray crystallographic data, which show that crystalline uncomplexed nonactin and tetranactin are relatively open and planar.[3-4]

Nonactin, which is less soluble than monactin or dinactin in CH_3OH, apparently is also the least effective at forming hydrogen bonds with CH_3OH, as evidenced by the relative lack of splitting of the 1725-cm^{-1} peak of nonactin in 4:1 (v/v) CH_3OH:$CHCl_3$ and the appreciable splitting of the 1725-cm^{-1} peak of monactin and dinactin in this same solvent (Figure 5). The greater ability of the higher nactins to form hydrogen bonds with methanol may be caused by the increased inductive effect of ethyl over methyl side chains, which would increase the dipole moment of the ester carbonyl groups.

Although the spectra of the uncomplexed nactins in solution are quite similar, spectra of the five crystalline nactins display numerous significant differences. This effect is especially pronounced in the 800–900-,

1200–1400-, and 2800–3000-cm^{-1} spectral regions. The disappearance of spectral differences between the nactins in solution implies that the differences observed in crystalline spectra are caused by contact forces between adjoining nactin molecules. Such forces couple and thereby split normal mode vibrations, and may also stabilize the molecule in a conformation not found in solution, thereby displacing backbone vibration frequencies. Although the minor chemical differences between the nactins are sufficient to have a substantial effect on their crystalline structure, the similarity of their solution spectra implies that such differences have little effect on the conformation of isolated nactin molecules in solution.

The spectra of crystalline nonactin, monactin, and tetranactin appear to comprise one family and those of dinactin and trinactin another family. Our findings thus agree with X-ray crystallographic studies, which have shown that nonactin and tetranactin form monoclinic crystals containing four molecules per unit cell,[3,4] while trinactin forms a triclinic crystal containing two molecules per unit cell.[28] The order–disorder structure of the nonactin crystal is reflected in the splitting of the ester carbonyl stretch line (1725 cm^{-1}); that this line is not split in the spectra of the other nactins suggests that replacement of even a single C^{10} methyl group with an ethyl group (as in monactin) is sufficient to stabilize the nactin crystal structure, eliminating the order–disorder structure found in nonactin. The spectroscopic data shown in Table I indicate that monactin probably has a crystal structure similar to tetranactin, while dinactin probably has a structure similar to trinactin.

Line assignments for the five nactins in the solid state and in CCl_4, $CHCl_3$, CH_3OH, and 4:1 (v/v) CH_3OH: $CHCl_3$ solution (Tables I–IV) are consistent with our previous work.[15,16] The methyl and methylene stretch and bend modes can be assigned in great detail. Extensive previous work on esters and polyesters supports our assignments of ester group modes, while line assignments for tetrahydrofuran ring vibrations are less certain.

Note added in proof: Kyogoku, Veno, and Akutsu. (*Biopolymers,* in press) have recently reported Raman, infrared, and NMR spectra of tetranactin and its KSCN complex in the solid state and in $CDCl_3$ solution. The mean conformation of the fluctuating framework of uncomplexed tetranactin in $CDCl_3$ solution is found to resemble the conformation of crystalline, uncomplexed nonactin (S_4 symmetry).

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