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F.T.-I.R. SPECTRA OF OLIGO- AND POLY-NUCLEOTIDES*,†

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ABSTRACT

Fourier-transform infrared (F.t.-i.r.) spectra of some synthetic mono-, oligo-, and poly-nucleotides and a natural DNA extracted from crab gonad have been recorded. Assignments of the observed frequencies are proposed by reference to our previous interpretation of laser-Raman and F.t.-i.r. spectra of constituents of nucleic acids. The spectra reflected structural differences between poly[d(A-T).d(A-T)] and crab gonad DNA (which is extremely rich in d(A-T) base-pairing). Such differences are mainly due to the effect of hydration on the conformational properties of natural DNA. Although the degree of complexity of the molecules investigated is higher than that of mononucleotides, most of the characteristic i.r. bands remain recognizable and assignable by comparison with the spectra of nucleic acid constituents previously studied.

INTRODUCTION

Infrared spectroscopy is recognized as one of the most powerful tools for investigating the structure of biomolecules. The recent advent of Fourier-transform infrared spectroscopy (F.t.-i.r.) has afforded detailed information at the molecular level through data processing of digitized spectra[2]. However, interpretation of F.t.-i.r. spectra implies knowledge of the chemical structure of the molecule studied and an approximate correlation of observed bands with functional groups. Both conditions necessary for the advantageous application of modern computerized i.r. techniques to the study of nucleic acids are provided by early work of Tipson[3,4].

The nature of the base, the nature and the ring structure of the sugar, the stereochemical disposition of the glycosidic linkage, and the position of the phosphate group in nucleotides were actively studied in a series of early papers by

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[*] F.t.-i.r. and Laser-Raman Spectra of Constituents of Nucleic Acids, Part VI; For Part V, see ref. 1.
[†] Dedicated to Dr. R. Stuart Tipson.

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TABLE I

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF GUANYLYL-(3′→5′)-ADENOSINE

D-*Ribose*	*Guanine*	*Adenine*	*Guanylyl-(3′→5′)-adenosine*	*Assignments*
	603			
628		622		
	648	642	644	A + G (breathing)
655				
		668		
	690		693	G
	704			
725	727	725	722	A[ν(C–C), ν(C–N)] + G + R [δ(C–C–O)]
750				
	780		781	G
	790	800		
804				
			811	P
	852	850		
866				
	880	874	875	A + G
890				
910		913	911	A[r(NH_2)] + R[ν(C–C)]
922				
		940		
950	950		948	A + G[δ(N–C=N) and δ(N–C–N)]
957				
			994	P
1016				
1035				
	1043		1055	G
1076			1075	R[δ(C–O–H)]
1086			1090	R
1116				
	1120	1126	1128	A[ν(C-2–N-1= C-6), ν(C-5–N-7=C-8)] + G [δ(C–N=C)]
1135				
1150	1150			
		1156		
1160				
1175	1175		1180	A + G[δ(C-8–H)]
1220	1216		1231	P
1245		1254		
	1264			
1280				
			1301	
		1310		
1315				
1320				
		1336	1335	A[ν(C–N), ν(C=N)]
1340				
1365				
	1375	1370		

Table I (continued)

D-*Ribose*	*Guanine*	*Adenine*	*Guanylyl-(3'→5')-adenosine*	*Assignments*
1380			1381	R
			1400	
1415				
	1420	1420	1420	A + G[δ(N-7=C-8-H)]
1440				
1455		1453		
	1465			
		1470		
	1480		1483	G[ν(N-7=C-8) and ν(C-8-N-9)]
		1510		
			1536	
	1566			
			1579	
		1606	1606	A[ν(C=N), ν(C=C)]
1635				
	1640		1644	G
			1650	
			1656	
	1676	1675		
			1694	
	1700			

[a] Key: δ = bending mode; ν = stretching mode; r = rocking mode; A = adenine (detailed assignments are given in ref. 8); G = guanine (detailed assignments are given in ref. 1); R = D-ribose (detailed assignments are given in ref. 7); P = phosphate.

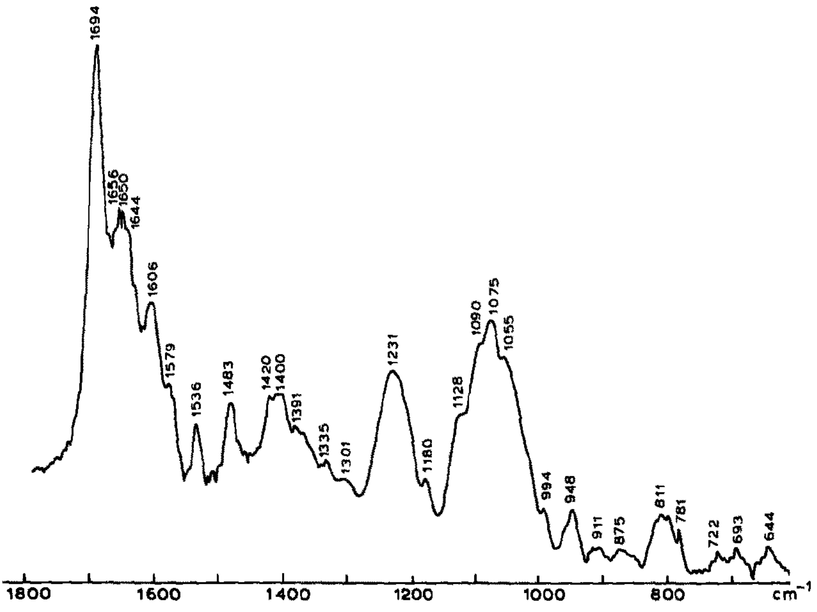

Fig. 1. F.t.-i.r. spectrum of solid guanylyl-(3'→5')-adenosine sodium salt.

Levene and Tipson[5]. One of the most extensive lists of i.r. bands and corresponding functional groups in carbohydrates and derivatives is also provided by Tipson[6]. We have recently discussed the vibrational spectra of the two sugars[7] of nucleic acids, and of adenine[8], thymine[9], cytidine[10], guanine[1] and their associated nucleosides, and have assigned most of the observed vibrations. Our objective was to use the interpretation of spectra of constituents of nucleic acids as a basis for analysis of the vibrational spectra of polynucleotides and nucleic acids. We now propose assignments of the observed F.t.-i.r. bands in the spectra of some mono-, oligo-, and poly-nucleotides and a natural DNA by comparison with the spectra previously studied[1,7-10].

EXPERIMENTAL

The F.t.-i.r. spectra of solid nucleotides were recorded with a Nicolet Fourier-transform infrared spectrometer Model 7199 for KBr pellets. Cytidine 5′-monophosphate, guanosine 2′-monophosphate, guanylyl-(3′→5′)-adenosine, cytidylyl-(2′→5′)-guanosine, poly[A] [poly(5′-adenylic acid)], poly[G] [poly(5′-guanylic acid)], poly[d(G-C)] [poly(deoxyguanylic-deoxycytidylic acid)] and poly[d(A-T).(d(A-T)] [poly(deoxyadenylicthymidylic acid)] were Sigma products. DNA from crab gonad was prepared by Guillé according to a previously published[11] technique. The range of frequencies explored was 1800–600 cm^{-1} and the wavenumbers are accurate to within ±2 cm^{-1}.

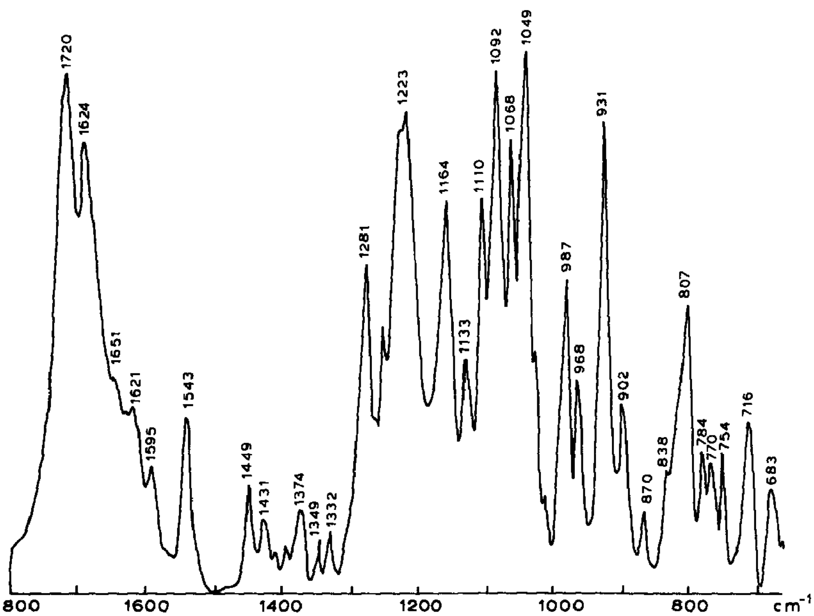

Fig. 2. F.t.-i.r. spectrum of solid cytidine 5′-monophosphate (free acid, crystalline).

RESULTS AND DISCUSSION

A. Synthetic nucleotides. — The F.t.-i.r. spectra of synthetic nucleotides are shown in Figs. 1–7. Assignments of the observed frequencies are proposed in Tables I–VII, respectively, for guanylyl-(3′→5′)-adenosine, cytidine 5′-monophosphate, cytidylyl-(2′→5′)-guanosine, poly[A], guanosine 2′-monophosphate, poly[G], and poly[d(G-C)]. In these tables are listed the observed frequencies for the sugar (D-ribose or 2-deoxy-D-*erythro*-pentose "2-deoxy-D-ribose"), the base and/or the nucleoside associated with the nucleotide investigated. Attribution of the observed frequencies to stretching or bending modes is denoted in the tables by R, D, A, C, G, Ad, Cy, Go when the vibration has as its origin respectively D-ribose, 2-deoxy-D-ribose, adenine, cytosine, guanine, adenosine, cytidine, or guanosine. The assignments are based on previously[1,7–10] discussed attributions of frequencies observed in the spectra of nucleic acid constituents. Importantly, most of the bands observed in the F.t.-i.r. spectra of nucleotides could be assigned by comparison with the preceding results[1,7–10]. However a slight shift towards higher frequencies, probably because of the constraint introduced by the bonds between monomers, is observed. It may be noted that the number of frequencies assignable to 2-deoxy-D-ribose (D) in the spectrum of poly[d(G-C)] (see Table VI) is higher than that of D-ribose (R) in the polyribonucleotide, poly[G] (Table V). Such a result is comparable to what has previously been noted for thymidine. Indeed, vibrations of the

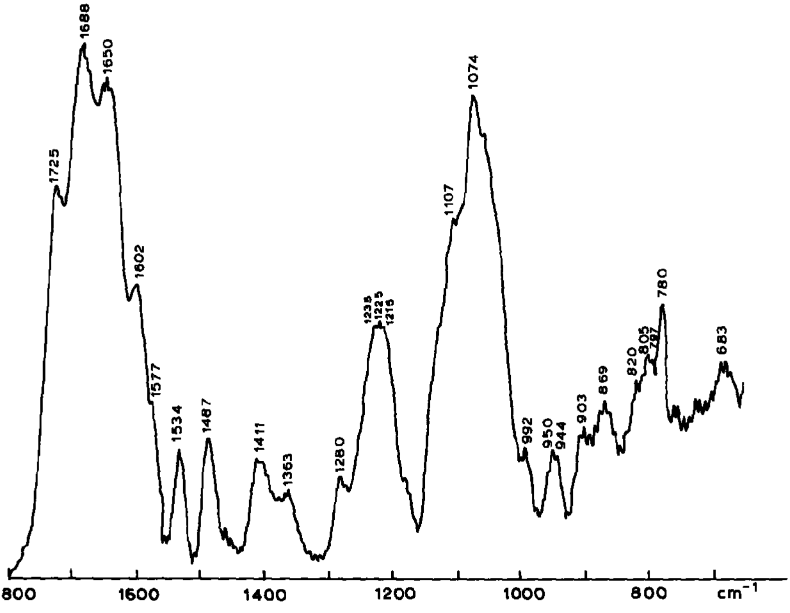

Fig. 3. F.t.-i.r. spectrum of solid cytidylyl-(2′→5′)-guanosine ammonium salt.

TABLE II

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF CYTIDINE 5′-MONOPHOSPHATE

D-*Ribose*	*Cytosine*	*Cytidine*	*Cytidine 5′-monophosphate*	*Assignments*
	602			
		621	621	Cy
628		630		
655		664		
			683	
	700			
		715	716	Cy
725				
		735		
750	758	757	754	R + C + Cy
			770	
	783		784	C[ν(ring)]
	794	790		
804			807	
	822	817		
		845	838	Cy
		855		
866		872	870	Cy
890				
			902	
910				
922				
			931	P
		943		
950				
957				
	966		968	C[ν(C-4–C-5)]
	995	984	987	C[δ(C-4–C-5–H)]
	1012			
1016				
1035		1034		
		1054	1049	Cy
1096			1068	R[δ(C–O–H)]
1086			1092	R
	1100	1102		
1116			1110	
1135		1137	1133	Cy + R
1150	1155	1154		
1160			1164	
1175				R
		1192		
		1213		
1220			1223	R
1245	1240	1248		
1280	1280		1281	R[τ(CH_2)] + C[ν(C-2–N-1) and ν(C-6–N-1)]
		1291		
1315		1309		
1320				
			1332	

Table II (continued)

D-Ribose	*Cytosine*	*Cytidine*	*Cytidine 5'-monophosphate*	*Assignments*
1340		1340	1349	R + Cy
1366		1366		
1380		1376	1374	C[δ(C=C–H)] + R + Cy
		1395		
		1403		
1415				
		1432	1431	Cy
1440				
1445			1449	
	1469	1463		
	1505	1500		
		1532		
	1540		1543	C(δ(N–H)]
		1605	1595	Cy
	1616		1621	C[ν(C-5=C-6)]
1635	1635			
		1648	1651	Cy
	1667	1660		
			1694	
	1705			
			1720	

[a] Key: δ = bending mode; ν = stretching mode; τ = twisting mode; C = cytosine; Cy = cytidine (for C and Cy, detailed assignments are given in ref. 10); R = D-ribose (detailed assignments are given in ref. 7); P = phosphate.

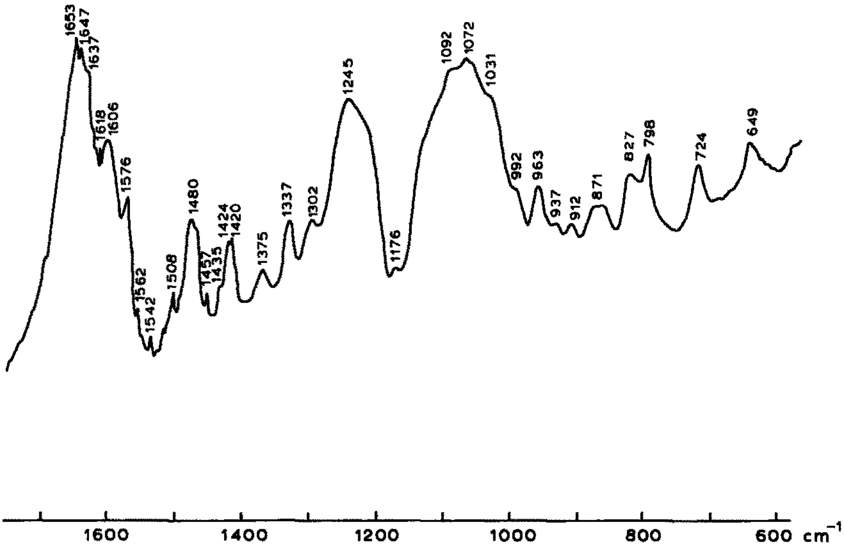

Fig. 4. F.t.-i.r. spectrum of solid poly[A] [poly(5'-adenylic acid), potassium salt, crystalline].

TABLE III

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF CYTIDYLYL-(2'→5')-GUANOSINE

D-*Ribose*	*Cytosine*	*Guanine*	*Cytidylyl-(2'→5')-guanosine*	*Assignments*
	602	603		
628				
		648		
655				
		690	683	G
	700			
		704		
725		727		
750				
	758			
	783	780	780	C[ν(ring)] + G
		790		
	794		797	C[breathing mode]
804			805	R[δ(C–C–O)]
	822		820	C[δ(N–H)]
		852		
866			869	R[ν(C–C)]
		880		
890				
			903	P
910				
922				
			944	
950		950	950	R + G[δ(N–C=N) and δ(N–C–N)]
957				
	966			
	995		992	
	1012			
1016				
1035				
		1043		
1076			1074	R[δ(C–O–H)]
1086				
	1100		1107	C[δ(N-1–C-6–H)]
1116		1120		
1135				
1150		1150		
	1155			
1160				
1175		1175		
		1216	1215	G[ν(C-2–NH_2)]
1220			1225	R
	1240		1235	C[ν(C-4–N-4)]
1245		1264		
1280	1280		1280	R + C[ν(C-2–N-1) and ν(C-6–N-1)]
1315				
1320				
1340				
1366	1366		1363	R[w(CH_2)] + C[δ(C=C–H)]
		1375		

Table III (continued)

D-*Ribose*	*Cytosine*	*Guanine*	*Cytidylyl-(2'→5')-guanosine*	*Assignments*
1380				
1415			1411	R
		1420		
1440				
1455				
		1465		
	1469			
		1480	1487	G[ν(N-7=C-8) and ν(C-8–N-9)]
	1505			
	1540		1534	C[δ(N–H)]
		1554		
		1566		
			1577	G[ν(C-2=N-3)]
			1602	
	1616			
1635	1635			
		1640		
			1650	G
	1667			
		1676		
			1688	G
	1705	1700		
			1725	G[ν(C=O), δ(NH_2)] + C

[a] Key: δ = bending mode; ν = stretching mode; w = wagging mode; G = guanine (detailed assignments are given in ref. 1); C = cytosine (detailed assignments are given in ref. 10); R = D-ribose (detailed assignments are given in ref. 7); P = phosphate.

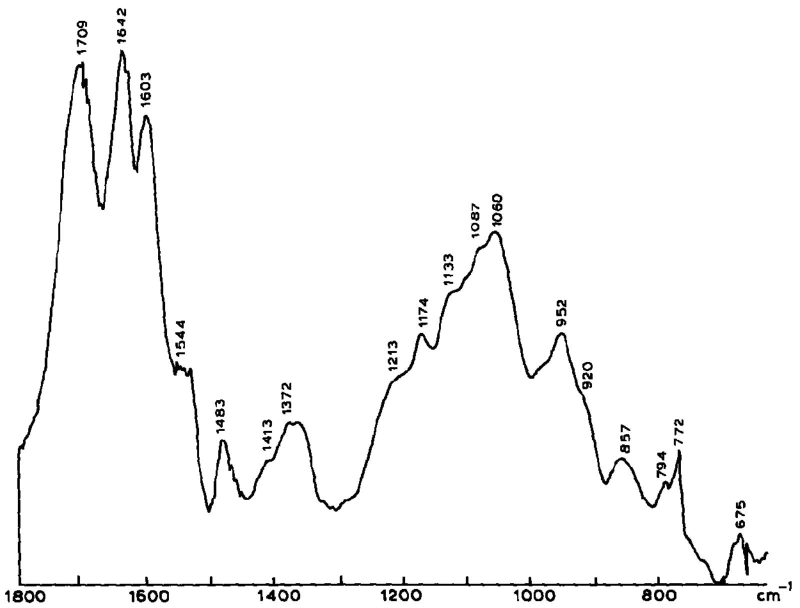

Fig. 5. F.t.-i.r. spectrum of solid guanosine 2'-monophosphate (disodium salt).

sugar residue remain at almost the same frequencies whereas the modes assignable to the base residue are shifted or masked.

Ribonucleosides and nucleotides. — Comparison of the F.t.-i.r. spectrum of cytidine 5′-monophosphate (Fig. 2) with that of cytidine[10] shows that most vibrations of the nucleoside are also observed for the nucleotide. When the phosphate group is at C-5′, the ring vibrations of the sugar are less affected than when this group is in the 2′ or 3′ position. The phosphate group at position 5′ is more free[12] and interacts with the guanine residue and the neighboring ribosyl groups in guanosine 5′-monophosphate (5′GMP). The phosphate group stabilizes the structure of 5′GMP by intramolecular hydrogen-bonding. On comparing the spectra of guanosine 2′-monophosphate (2′GMP) (see Fig. 5) and guanosine (ref. 1), it may be observed that the bands assigned to the sugar[1] in the frequency range 1300–900 cm^{-1} appear only as shoulders in the i.r. spectrum of 2′GMP. The principal vibrations of the sugar–phosphate backbone are observed[13] in the 1350–950 cm^{-1} region. Overlapping of the two bands from the phosphate group in this region of the spectrum, and the possibility of intramolecular H-bonding (especially between the 2′-phosphate group and 3′-OH), contributes to broadening of absorptions and absence of the exocyclic vibrations from D-ribose. The phosphate vibration was localized[13] at 1230 cm^{-1} in the spectrum of yeast RNA and was supposed to originate from interactions of the phosphate group with the folding of the helix and from hydrogen bonding with such neighboring groups as the 2′-OH group of D-ribose. Such binding leads to a stabilized structure of the macromolecule.

Inspection of the spectra of the mononucleotide 2′-GMP (Fig. 5) and the polynucleotide poly[G] (Fig 6) reveals that the bands in the 1300–900 cm^{-1} frequency region are more broad and overlapped for the mononucleotide. This could be because the phosphate group is in the 5′-position in poly(guanylic acid) (poly[G]). Indeed, in the 5′-position, the phosphate group interacts with guanine rings and the adjacent ribose group, stabilizing the structure of the polynucleotide. Besides symmetric and antisymmetric bending of the PO_2 group at 1245 and 1068 cm^{-1}, respectively, vibrations of the nucleoside constituent (guanosine) of poly[G] are found at frequencies (see Table V) comparable to those observed[1] in the i.r. spectrum of pure guanosine.

Likewise, the spectrum of poly(adenylic acid) (poly[A]) (Fig. 4) shows a number of absorptions similar to those of adenine[8]. Such a resemblance was also observed[14] on comparison of the i.r. spectra of AMP (adenosine 5′-monophosphate) and poly[A] in aqueous solution. The Raman spectra of adenosine and AMP in solution were found[15] to be very similar, except for a band at 980 cm^{-1} assigned to phosphate stretching. The sugar–phosphate backbone gives rise to weak and broad Raman lines in the 1100–800 cm^{-1} frequency region in the spectra of cytosine derivatives[15]. When the ribose–phosphate–ribose chain links two different bases as in cytidylyl-(2′→5′)-guanosine (Fig. 3) or guanylyl-(3′→5′)-adenosine (Fig. 1), the phosphate groups are readily characterized by two absorptions at about 1230 and 1075 cm^{-1} of relatively strong intensities. Such a result is probably due to the fact

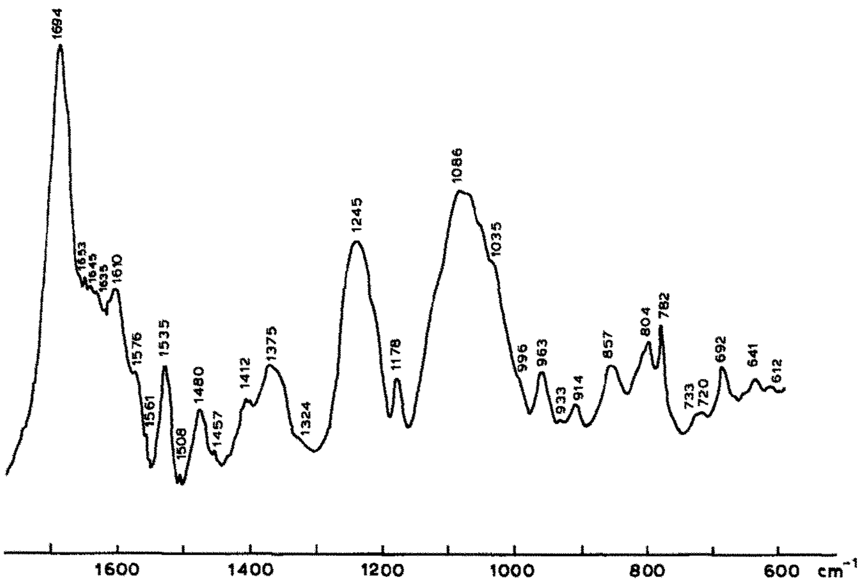

Fig. 6. F.t.-i.r. spectrum of solid poly[G] [poly(5′-guanylic acid) potassium salt, lyophilized].

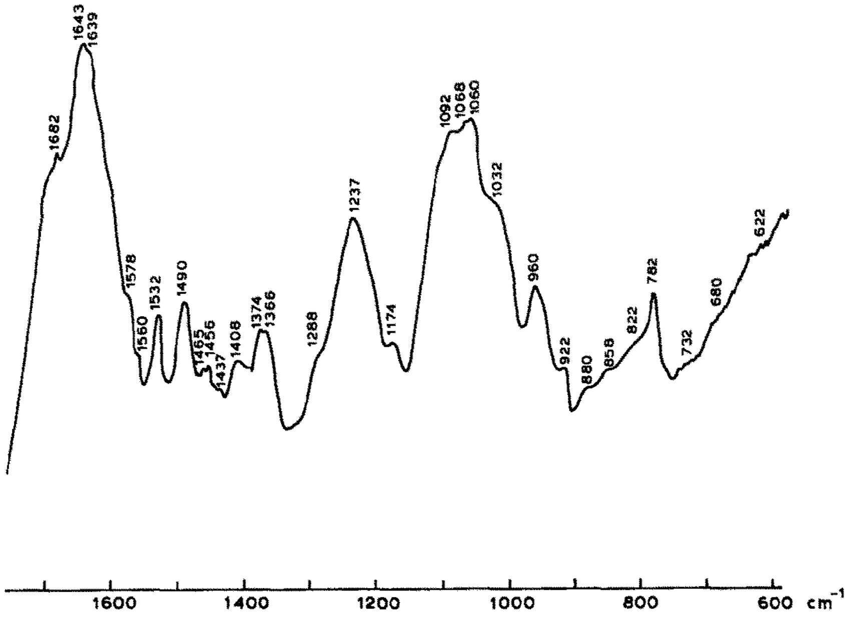

Fig. 7. F.t.-i.r. spectrum of solid poly[d(G-C)] (polydeoxycytidylic acid, sodium salt).

TABLE IV

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF POLY[A]

D-*Ribose*	*Adenine*	*Adenosine*	*Poly*[*A*]	*Assignments*
			539	
	622			
628				
	642	638		
655			649	R[δ(O-4–C-1–O-1)]
		665		
	668	670		
		705		
725	725	720	724	A[ν(C–C), ν(C–N)] + R [δ(C–C–O)]
750				
		768		
	800	795	798	A[ν(C–C)]
804				
		820		
			827	P
		842		
	850			
		858		
866				
	874		871	A
890				
		902		
910	913		912	A + R[ν(C–C)]
922				
	940		937	A[δ(N–C=N)]
950				
957				
		965	963	Ad
		977		
			992	P
		1010		
1016				
1035		1035	1031	R + Ad
		1055		
		1070	1072	Ad
1076				
1086		1090	1092	Ad
		1107		
1116				
	1126	1126		
1135		1140		
1150				
	1156			
1160				
1175		1178	1176	R + Ad
		1208		
1220		1224		
1245	1254	1250	1245	R + A[ν(C–NH_2)]
1280				
		1302	1302	Ad
	1310			

Table IV (continued)

D-*Ribose*	*Adenine*	*Adenosine*	*Poly*[*A*]	*Assignments*
1315				
1320				
1340	1336	1333	1337	R + A[ν(C–N), ν(C=N)]
		1353		
1365	1370	1370	1375	R + A[δ(C-8–H), δ(C-2–H)]
1380				
		1388		
1415	1420	1415	1420	R + A[δ(N=CH)]
		1425	1424	Ad
1440			1435	R
1455	1453		1457	R[δ(CH_2)] + A[ν(imidazole ring)]
	1470	1475	1480	A
	1510	1505	1508	A
			1542	
			1562	
		1573	1576	Ad
	1606	1605	1606	A[ν(C=N), ν(C=C)]
			1618	
1635			1637	R[δ(H_2O)]
			1647	
			1653	
	1675	1665		

[a] Key: δ = bending mode; ν = stretching mode; A = adenine; Ad = adenosine (for A and Ad, detailed assignments are given in ref. 8); R = D-ribose (detailed assignments are given in ref. 7); P = phosphate.

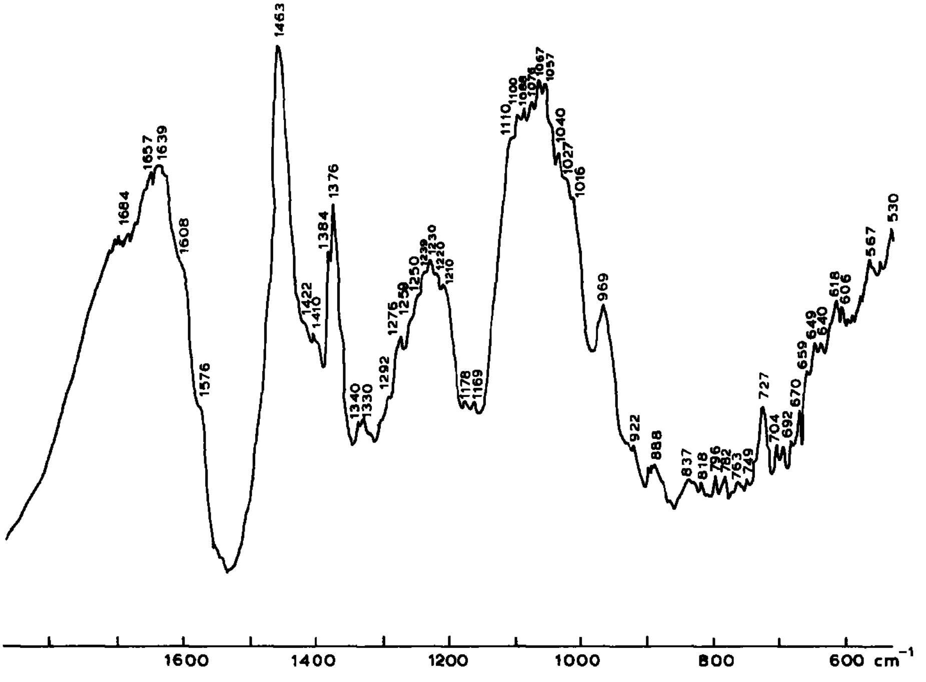

Fig. 8. F.t.-i.r. spectrum of solid poly[d(A-T).d(A-T)] [poly(deoxyadenylic-thymidylic acid), sodium salt].

TABLE V

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF POLY[G]

D-*Ribose*	*Guanine*	*Guanosine*	*Guanosine 2'-mono-phosphate*	*Poly*[*G*]	*Assignments*
	603				
		610			
				618	G
628					
	648			641	G (breathing)
655		655			
		670			
		675	675		
	690	692		692	G
	704	700			
		708			
		718		720	Go
725	727			733	G + R[δ(C–C–O)]
		740			
750		752			
			772		
	780	780		782	G
	790		794		
804		800		804	R[δ(C–C–O)]
		826			
	852	860	857	857	G[ν(C–C)]
866					
	880	885			
890					
	900				
910				914	R[ν(C–C)]
922		922	920		
				933	P
950	950		952		
957				963	R
		1005		996	Go
1016					
	1025				
1035				1035	R
	1043	1045			
		1053			
		1065	1060		
1076					
1086		1085	1087	1086	R
1116	1120				
1135		1135	1133		
1150	1150				
1160					
1175	1175	1182	1174	1178	G[δ(C-8–H)] + R
	1216	1210	1213		
1220					
		1228			
1245		1250		1245	R
	1264	1262			

Table V (continued)

D-*Ribose*	*Guanine*	*Guanosine*	*Guanosine 2'-monophosphate*	*Poly*[*G*]	*Assignments*
1280		1280			
1315					
1320		1325		1324	R[w(CH_2)]
1340		1340			
		1355			
1365					
	1375		1372	1375	G[δ(C-8-N-H), δ(C-8-H), ν(C-8-N)]
1380					
		1400			
1415			1413	1412	R
	1420				
		1430			
1440					
1455				1457	R[δ(CH_2)]
	1465				
	1480	1490	1483	1480	G[ν(N-7=C-8) and ν(C-8-N-9)]
				1508	
		1540	1544	1535	Go
1554				1561	G[ν(C=C)]
	1566	1570		1576	G[ν(C-2=N-3)]
			1603		
		1615		1610	Go
1635		1630		1635	R[δ(H_2O)]
	1640	1645	1642	1645	G
				1653	
	1676	1675			
				1694	
	1700		1709		
		1736			

[a]Key: δ = bending mode; ν = stretching mode; G = guanine; Go guanosine (for G and Go, detailed assignments are given in ref. 1); R = D-ribose (detailed assignments are given in ref. 7); P = phosphate.

that when phosphate groups are strongly held by covalent bonds, the bands corresponding to their vibrations are well resolved and have less influence on the rest of the spectrum.

2-Deoxyribopolynucleotides.—The F.t.-i.r. spectrum of poly[d(G-C)] is shown in Fig. 7. It was expected that the vibrations of 2-deoxyribose should be detectable in Fig. 7. However comparison of Fig. 7 with previous results on 2-deoxy-D-ribose[7] leads to the conclusion that phosphate vibrations in the 1300–900-cm^{-1} range mask the characteristic i.r. bands of the sugar. Even CH_2 deformations in sugar, corresponding to bands in the 1500–1300-cm^{-1} region, have relatively weak to moderate intensities. This is probably because the helical structure of poly[d(G-C)] allows a higher freedom to the vibrations from the deoxy sugar, causing frequency shifts

TABLE VI

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF POLY[G-C]

2-Deoxy- D-*ribose*	*Guanine*	*Cytosine*	*Poly*[*d*(*C-G*)]	*Assignments*
			529	
			570	
	603	602		
			622	
633				
	648			
672			680	D
686	690			
	704	700		
	727		732	G
740				
760		758		
770				
	780	783	782	G + C[ν(ring)]
	790	794		
815		822	822	C[δ(N–H)] + D[ν(C–C)]
	852		859	G[ν(C–C)]
880	880		880	G + D
896				
926			922	D
	950			
		966	960	C[ν(C-4–C-5)]
985				
		995		
		1012		
1017				
			1032	P
1045	1043			
			1060	P
			1068	P
1092			1092	D
		1100		
1116				
	1120			
1152	1150	1155		
	1175		1174	G[δ(C-8–H)]
1200				
	1216			
1236		1240	1237	C[ν(C-4–N-4)] + D
1260				
	1264			
1280		1280	1288	C[ν(C-2–N-1) and ν(C-6–N-1)] + D
1303				
1344				
1350				
1370		1366	1366	C[δ(C=C–H)] + D
	1375		1374	G[δ(C-8–N-H), δ(C-8–H), ν(C-8–N)]
1390				
1415			1408	D
	1420			

Table VI (continued)

2-Deoxy-D-ribose	*Guanine*	*Cytosine*	*Poly[d(C-G)]*	*Assignments*
1440			1437	D
			1456	
1470	1465	1469	1465	C[ν(C-4-N-3), ν(C-2-N-3)] + G[δ(C-N-H)] + D
	1480		1490	G[ν(N-&=C-(), ν(C-8-N-9)]
		1505		
		1540	1532	C[δ(N-H)]
	1554		1560	G[ν(C=C)]
	1566			
			1578	
		1616		
1635	1640	1635	1639	C + G + D[δ(H_2O)]
			1643	C + G + D
			1649	
		1667		
	1676		1682	
	1700	1705	1696	C[δ(NH_2)] + G[ν(C=O), δ(NH_2)]

[a]Key: δ = bending mode; ν = stretching mode; C = cytosine (detailed assignments are given in ref. 10); G = guanine (detailed assignments are given in ref. 1); D = 2-deoxy-D-*erythro*-pentose (detailed assignments are given in ref. 7); P = phosphate.

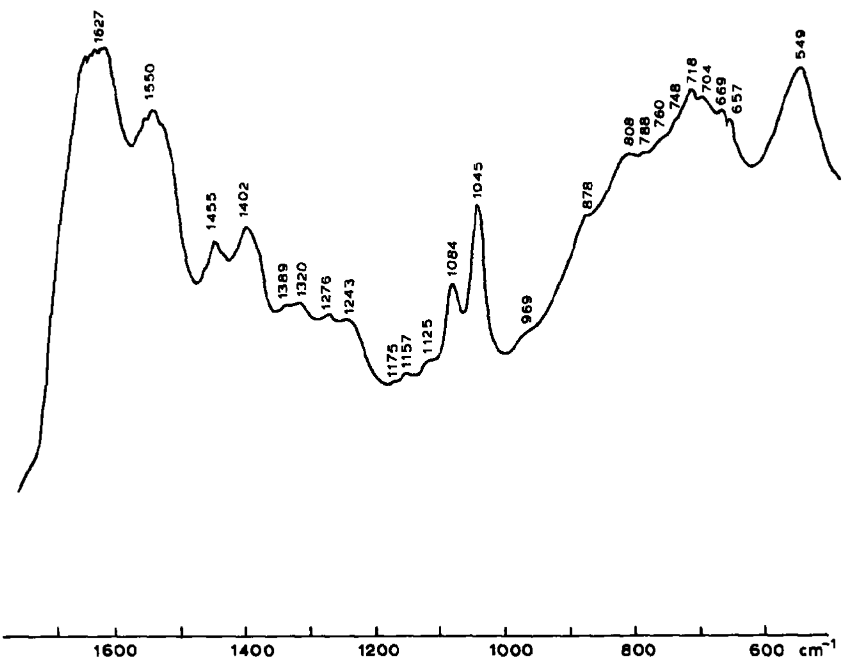

Fig. 9. F.t.-i.r. spectrum of crab DNA.

TABLE VII

BANDS OBSERVED[a] IN THE F.T.-I.R. SPECTRUM OF POLY[d(AT).d(AT) AND THE CRAB DNA

2-Deoxy-D-ribose	*Adenine*	*Thymine*	*Poly[d(AT).d(AT)]*	*Crab DNA*	*Assignments*
	622	616	618		A[δ(N–C–C)] + T [δ(N–C–C)]
633					
	642		640		A
			649		
		659	657		
672	668		670	669	A + D
686			692		R
			704	704	
	725		727	718	A[ν(C–C), ν(C–N)]
740		740		743	T[δ(C–H)] + D
			749		
760		760	763	760	T[ν(C-5–CH_3)]
770					
			782	788	
	800		796	808	A[ν(C–C)]
815		814	818		T[ν(C-4–C-5)] + D [ν(C–C)]
		845	837		T[δ(N–H)]
	850				
	874			878	A
880			888		D
896					
	913				
926			922		D
		935			
	940				
			969	969	P
985		983			
1017			1016		D
	1025	1030	1027		A[δ(C–N–C)] + T [δ(C–N–H)]
1045			1040	1045	D
		1050	1057		T[δ(N–C–H)]
			1067		P
			1076		P
1092			1088	1084	D
			1100		P
1116			1110		D
	1126			1125	A[δ(C-2–N-1=C-6) and δ(C-5–N-7=C-8)]
1152	1156	1152		1157	A[δ(C–H) + T[r(CH_3)]
			1169		
			1178	1175	
1200		1204			
		1215	1210		T[ν(C–N)]
			1220		P
1236			1231		D
		1246	1239	1243	T[δ(C-5=C-6–H)]
	1254		1250		A[ν(C–NH_2)]

Table VII (continued)

2-Deoxy-D-ribose	*Adenine*	*Thymine*	*Poly[d(AT).d(AT)]*	*Crab DNA*	*Assignments*
1260		1260	1259		T[ν(C–N)] + D
1280			1276	1276	D
			1292		
1303	1310		1308		A[ν(C–N), ν(C=N)] + D
	1336		1330		A[ν(C–N), ν(C=N)]
1344			1340	1339	D
1350					
1370	1370		1376		A[δ(C-8–H), δ(C-2–H)] + D
		1382	1384		T[$\delta(CH_3)$]
1390					
				1402	
		1410	1410		T
1415					
	1420		1422		A[δ(N=CH)]
		1430			
1440		1448			
	1453			1455	A ν[imidazole ring]
1470	1470		1463		A + D
		1482			
		1492			
	1510				
				1550	
			1576		
	1606		1608		
1635			1639	1627	A
			1657		
	1675	1680	1684		A[$\delta(NH_2)$] + T [ν(C-5=C-6)]
			1700		
		1735			

[a] Key: δ = bending mode; ν = stretching mode; r = rocking mode; A = adenine (detailed assignments are given in ref. 8); T = thymine (detailed assignments are given in ref. 9); D = 2-deoxy-D-*erythro*-pentose (detailed assignments are given in ref. 7); P = phosphate.

and lowering of intensities. One of the most characteristic and intense vibrations of 2-deoxyribose, located at 815 cm^{-1} in the i.r. spectrum and 812 cm^{-1} in the Raman spectrum[8], is shifted towards lower frequency (782 cm^{-1}) in the spectrum of poly[d(G-C)] (see Fig. 7 and Table VI).

It is known[16] that left-handed Z-structure may be induced in DNA and polynucleotides containing alternating G-C base-pairing sequences. In the Z-structure, the glycosidic linkage ($\chi_{C\text{-}N}$) of guanine bases are in the anti-disposition about the glycosidic linkage and have the C-2'endo (or C-3'endo) puckering for the sugar[17–23]. Such changes in conformation could account for the shifts of frequencies observed in the F.t.-i.r. spectrum of poly[d(G-C)] (Fig. 7). These comments on the i.r. spectra (Fig. 1–7) of mono-, oligo-, and poly-nucleotides attempt to show that detailed interpretation of the spectra of simple constituents of nucleic acids helps in understanding the structural features manifested in the spectra of polynucleotides and nucleic acids. The F.t.-i.r. data should also be correlated with that from other physicochemical techniques (calorimetry, viscometry, n.m.r. spectroscopy, crystallography, u.v. spectroscopy) in order to consolidate the conformational interpretations.

B. Synthetic and natural DNA. — The F.t.-i.r. spectra of poly[d(A-T).d(A-T)] and crab DNA are shown in Figs. 8 and 9, respectively. The difference in background between the two spectra is probably attributable to the hydration of the natural DNA. The spectrum of crab DNA was compared with that of poly[d(A-T.d(A-T] because it has been observed[24] that DNA isolated from crab gonad is extremely rich (~80%) in d(A-T) base-pairing.

The frequency region above 1500 cm^{-1}, which is certainly influenced by the binding of water, shows an intensity maximum at 1630–1640 cm^{-1}, comparable in width and intensity in both spectra. The presence of liquid water and base pairs other than (A-T) (~20%) are at the origin of the differences between the two spectra. However, it may be observed (Table VII) that most of the frequencies observed in the crab DNA spectrum are found in the spectrum of poly[d(A-T).d(A-T)] and could be assigned to A, D, T, or P, the major constituents of the polynucleotide. Hydrogen bonding is generally more important in the presence of water. It is at the origin of the B-structure of DNA and contributes in enhancing the stability of the double helix[25]. However, such weak interactions as water–water and water–nucleic acid binding generally lead to broadening of bands and shifts in frequencies. All vibrations of groups capable of binding to water are affected by hydration. It is the case for phosphate groups, which give rise to two major bands at 1230 and 1067 cm^{-1} in the spectrum of the synthetic polynucleotide (Fig. 8), whereas only one band is observed in the spectrum of crab DNA (Fig. 9). The general appearance of the spectrum of poly[d(A-T).d(A-T)] between 1300 and 900 cm^{-1} recalls the spectra of other synthetic nucleotides (see Figs. 1–7).

Another frequency region affected by hydration of DNA is the local-symmetry region (1300–1500 cm^{-1}), where CH_2 vibrations are found. The difference between spectra of poly[d(A-T).d(A-T)] and DNA mainly concerns the intensities of frequencies observed at 1398, 1402, and 1455 cm^{-1}. Hydration was found[26] to be more important in the monolayer directly linked to A-T sequences than it is to G-C base-pairing sequences. Variation in base-pair composition along the polynucleotide chain gives rise to differences in local potentials, which are at the origin of differences in water association to the helix grooves[26].

CONCLUSION

Comparison of the F.t.-i.r. spectra of polynucleotides with Raman and i.r. spectra of their monomeric constituents permitted assignments of the observed frequencies. Structural modifications caused by puckering of the sugars or by intramolecular binding of phosphate groups give rise to shifts of frequencies and decreases in intensities of the observed bands. Comparison of the i.r. absorptions from a synthetic and a natural DNA clearly shows the important influence of hydration on the vibrations of groups of polar atoms. Although the environment of the vibrating tensors changes when the degree of complexity of the molecule increases, most of the characteristic i.r. bands remain recognizable and assignable by comparison with the spectra of pure monomeric compounds.

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