THE INFRARED SPECTRA OF SOME DNP-α-AMINO ACIDS[1, 2]

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ABSTRACT

The infrared spectra of some DNP-α-amino acids were examined using the KBr technique in the region 5000 to 625 cm^{-1}. There is sufficient variation in spectra of closely related DNP-amino acids to allow positive identification even though the spectra on the whole are more strikingly similar than they are different. Differences in varying degrees were also noted between L- and DL-forms.

INTRODUCTION

The DNP[4]-derivatives of amino acids recently have assumed importance in amino acid sequence studies on peptides and proteins (1). While the KBr pellet spectra of a large number of the amino acids (2, 3), and also of their 3-phenyl-2-thiohydantoins (4), are reported in the literature, there is little information with regard to the infrared spectra of DNP-derivatives.[5] This study was undertaken to compare the spectra (*a*) of the DNP-derivatives of amino acids with the spectra of the corresponding free amino acids as given in the literature, (*b*) of the DNP-derivatives of comparable L- and DL-amino acids, and (*c*) of the DNP-derivatives of structurally related acids, to see whether such compounds can be identified by their spectra.

EXPERIMENTAL

The spectra were taken on a Model No. 21 Perkin–Elmer spectrophotometer (equipped with sodium chloride optics). Pellets were made by first mixing the DNP-amino acids with KBr in an agate mortar, and then pressing the material in the Perkin–Elmer die, under evacuation. The DNP-amino acids had been prepared in this laboratory by the method of Levy and Chung (5), and each was crystallized several times and found pure by paper chromatography. Melting points for all derivatives were determined and are essentially in agreement with the values given in literature (6).

DISCUSSION

Table I lists and characterizes the spectral absorption bands in the 5000 to 625 cm^{-1} region. As a result of dipolar ion structure many amino acids possess a characteristic absorption frequency at about 1587 cm^{-1} which is related to the COO^- group, as well as a relatively weak absorption at about 2128 cm^{-1} which may be attributed to NH frequencies in the $—NH_3$ ion. The absence of this absorption peak at 2128 cm^{-1} in the case of DNP-derivatives of amino acids, where the amino group is attached to the dinitrophenyl ring, was therefore expected.

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[2]*Spectra deposited with Documentation of Molecular Spectroscopy, Butterworth Scientific Publications, London, England.*
[3]*Work done during tenure of Lederle Medical Faculty Award.*
[4]*DNP is used as an abbreviation for 2,4d-initrophenyl.*
[5]*The first systematic study of the vibrational spectra of α-amino acids was made by Edsall and his co-workers (J. Phys. Chem.* ***41****, 133 (1937); J. Chem. Phys.* ***4****, 1 (1936);* ***5****, 225, 508 (1937); J. Am. Chem. Soc.* ***65****, 1767 (1943);* ***71****, 474 (1950); and more recently J. Am. Chem. Soc.* ***80****, 3807, 3813, 3818, 3823, 3827 (1958). This work is concerned primarily with Raman spectra in aqueous solutions. The first infrared studies were published by Freyman and his co-workers (J. phys. radium,* ***7****, 30 (1936)).*

TABLE I

Absorption spectra in the infrared in the 5000–625 cm^{-1} region[a]

DNP-L-arginine 245–250 (*d*)[b]	3311sh 3236SB		2882MB			1658SB 1618SB	1580SB 1570sh	1520SB	1484SS 1464sh 1451sh	1408SB	1387SS	1335SB
DNP-L-alanine 182	3311MS	3058MB			1718SS	1675MB 1623SS	1590SS	1522SS 1502MS	1464WB	1414SB	1359SB	1333sh
DNP-DL-alanine 180.5	3279MS	3058MB			1709SS	1618SS	1587SS	1522SS 1499MS	1464WB	1414SB	1364sh	1333SB
DNP-L-aspartic 186–187	3279MS	3058MB	2857MB		1712SB	1618SS	1590SS	1546MS 1522SS 1499SS		1425SB 1395SS	1361SB	1333SB
Bis-DNP-L-cysteine 155–158	3279MS	3058MB	2907WB		1748SS	1613SB	1590SB	1515SB 1511sh		1427MB	1385MB	1333SB
Di-DNP-L-cystine 118–121	3311sh 3289MS				1745MB	1616SS	1590SS	1520SB		1422MB		1333SB
DNP-glycine 209	3311MS				1712SS	1608SS	1595sh	1517SB 1497SS	1447MS 1441sh	1422sh 1412SB	1362SS	1335SB
Di-DNP-L-histidine 219	3268MS	3058MB			1715MB	1618sh 1613SB	1582SS	1511SB		1416SB 1412sh	1362MS 1359sh	1333sh 1326SB 1321sh
DNP-hydroxy-L-proline 174–178	3425SB 3319sh	3095WB	2950WB		1744SB	1610SB	1588SB	1533SB 1512SB	1464vw 1439MB 1450sh		1384sh	1345SB
DNP-L-isoleucine 109–110	3289MS	3058MB	2924MB	2538vwB	1712SS	1618SS	1587SS	1515SB	1456MB	1412SS	1364MS	1333SB
DNP-DL-isoleucine 174.5	3289WS	3030MB	2915MB 2833sh		1712SS	1621SS	1582SS	1517MS 1502MS	1453WB	1412SS	1377WS 1361MS	1332SB
DNP-L-leucine 102	3289SS	3030SB	2899SB		1712SB	1618SB	1587SB	1513SB 1495SB	1464SB	1422SB	1385SS 1364SS	1325SB

TABLE I (*Continued*)

Absorption spectra in the infrared in the 5000–625 cm^{-1} region[a]

DNP-L-arginine 245–250 (*d*)[b]	1277SB	1250SB		1176sh	1139SB	1094MB		1054MB				948vw	919MB	
DNP-L-alanine 182	1302MS	1261SB 1230SB	1217MB	1157SS	1140MB	1124MB	1072WB	1057MS				940WB	919WB	
DNP-DL-alanine 180.5	1292SB 1284sh	1242SB 1232sh		1172MB 1156MS		1119SS		1053MB					916MB	
DNP-L-aspartic 186–187	1282SB	1229SB	1220sh	1151SS	1136SS	1109SB		1056SS	1040MB 1037sh			928SB	915MB	905SB
Bis-DNP-L-cysteine 155–158	1290SB	1244SB 1239sh		1171MB	1143SB 1135sh	1107SB 1100sh		1050MB					918MB 916sh	902WB
Di-DNP-L-cystine 118–121	1299sh 1292SB				1140sh 1135MB	1105MB		1058MB					926MB	
DNP-glycine 209	1307SB 1290SB	1250sh 1232SB		1155SS	1131SS	1105SS		1056MB		996MS	978WB	945MB	922SS	
Di-DNP-L-histidine 219	1285sh	1230SB	1222sh		1144SB 1133SB	1103SB		1054MB	1026MB	996SB	971MB	952MB 936MB	918MB	
DNP-hydroxy-L-proline 174–178	1285sh	1245WB	1210vw	1183MB	1152MS	1125MS	1074MS			997MS	980MS		927WS	903vWB
DNP-L-isoleucine 109–110	1285SB	1244SB	1222SB	1163sh	1147SS 1134SB	1121MB 1101MB		1056MB			966WB		921MB 919sh	
DNP-DL-isoleucine 174.5	1290SS	1261sh 1247SB 1232sh 1229sh			1149SS	1124MS 1098MB		1054WS		1008vw	965WB		918MB	
DNP-L-leucine 102	1302MB 1276SB	1261SB 1232SB	1206SB	1151SS	1139WB 1125SB	1094SB	1076SB	1054SB			958MB	939SB	917SB	

DNP-L-arginine 245–250 (*d*)[b]			831MS	811MB		763MB	743SB	722MB					645MB	
DNP-L-alanine 182			833WB 828WS			765WB	743MB	720WB			667WB			
DNP-DL-alanine 180.5			832WB	817WS		763WB	743MB	724WB 720sh						
DNP-L-aspartic 186–187		858WB	832MS	807MS	785WB	762WB	744SS[1]	716SB	687WB	680WB	678WB	658WB	644WB	
Bis-DNP-L-cysteine 155–158	877WB		833MS	819MB		762WB	745MB 732MS	718MB				660WB		
Di-DNP-L-cystine 118–121			832MB				743MB							
DNP-glycine 209	889WB 886sh		835MS	818SS		762WS	745SS	714WB	695WB	684WB		657MB		
Di-DNP-L-histidine 219	885WB 879vw	870WB	832MB	822MB 818sh		759WB	743MS	719MB						
DNP-hydroxy-L-proline 174–178	882vwB		832vwS	810SS		758WS	743SS	724vwB		689MB		653MB		
DNP-L-isoleucine 109–110			831MS 826MB	821MB		765WB	743MS 733MB	719WB						
DNP-DL-isoleucine 174.5			830WS	818MS	791vw	760vw	743MB	717WB	690vw		667vw		644WB	
DNP-L-leucine 102			831SB	817SB	774MB	767MS	741SS	711SB	699WB	685MB	665MB			

TABLE I (*Continued*)

Absorption spectra in the infrared in the 5000–625 cm^{-1} region[a]

DNP-DL-leucine 132–133	3322WS		3058WB		2899MB					1712SS	1618SS	1592SS
Di-DNP-L-lysine 178–180	3300WS		3058WB		2890WB					1715MS	1618SS	1587SS
E-DNP-L-lysine 197–200	3333MS				2841SB					1724MS	1618SS	1587SS
DNP-DL-methionine 122	3279MS		3058MB		2874MB					1718SB	1618sh 1608SS	1590SS
DNP-L-phenylalanine 191	3279SS 3247sh	3195sh 3175sh	3077sh		2882sh		1965WB	1821WB	1742SS	1715sh 1701sh	1618sh 1608SS	1580SS
DNP-DL-phenylalanine 219	3279MS 3247sh	3205sh 3185sh 3145sh	3058sh	3003sh			1980WB	1818WB	1742SS	1701sh 1686sh	1613SS	1580SS
DNP-L-proline 138	3356WB		3049MB		2833MB					1712SS	1603SS	1577SS
DNP-DL-serine 197–201	3344SS 3300SS		3077sh	3021sh 2959sh 2941sh	2899SB				1757SS		1616SS	1592sh 1587SS
DNP-DL-threonine 178–179	3390SB 3333SS		3086MS		2899SB				1757SS		1618SS	1587SS
DNP-L-tryptophan 217–221 (*d*)	3401SS 3322MS		3096MB		2915MB					1715SS	1613SS	1582SS
Di-DNP-L-tyrosine 184 (*d*)	3390sh 3279WB		3058WB								1613SS	1582SS
Di-DNP-DL-tyrosine 207 (*d*)	3378MS 3279MS		3058WB							1715SS	1616SS	1582SS
DNP-L-valine 132–133	3311SS	3215SS 3145sh		2950SS	2915SS				1748SS		1618SS 1616sh	1587SS
DNP-DL-valine 187–189	3279MS			3003MvB	2924MB 2890sh	2571WB				1712SS	1623SS	1587SS

TABLE I (*Continued*)

Absorption spectra in the infrared in the 5000–625 cm^{-1} region[a]

DNP-DL-leucine 132–133		1517SS	1499MS	1464WS		1422SS	1387vw	1355MS	1332SB	1302MB	1277MB	1261SB	1222MS	
Di-DNP-L-lysine 178–180		1517SS				1416SS			1337SB	1307MB	1269MB		1229MB	
E-DNP-L-lysine 197–200		1522SB				1420SS		1361MS	1330SB	1312SB			1238SB	1195SB
DNP-DL-methionine 122	1541WB	1513SS	1499SB			1420SB		1357MS	1330SS	1307SB	1274SB	1266sh	1244SB	1198MB
						1416sh							1229sh	
DNP-L-phenylalanine 191	1534WB	1515SS	1488SS	1451MS	1437sh	1420sh	1401SB	1362MS	1325SB	1307sh			1236SB	1203sh
							1395sh							
DNP-DL-phenylalanine 219	1538WB	1517SS	1486SS	1451WB	1439sh	1420MB	1393SB	1366MS	1330sh	1314sh	1287sh		1245SS	1205MB
									1321sh	1300sh	1282sh		1235SB	
									1318SS	1294SS				
DNP-L-proline 138		1520SB	1495SS		1447MS	1420MB	1377SS		1326SB	1297sh	1274sh		1221MB	
										1290SB				
DNP-DL-serine 197–201		1513SS	1495SS	1456MS	1431sh	1412SS		1362SS	1335SB	1316SB	1279sh	1266SS	1232SB	1198SB
DNP-DL-threonine 178–179		1520SS	1495SB			1420SB	1395sh	1364SB	1335SS	1316SB	1277SB		1235SB	1206SB
							1389sh	1357sh						
							1381MB							
DNP-L-tryptophan 217–221 (*d*)	1531MS	1515SS	1493MB	1449MS		1418SB		1361sh	1332SB	1290SB	1276SB		1250SB	1198SB
													1227SB	
Di-DNP-L-tryosine 184 (*d*)	1527SB		1502MS			1412MB			1340SB			1266SB		
			1479MS											
Di-DNP-DL-tryosine 207 (*d*)	1531sh	1517SS	1490SS		1439sh	1416SS		1362MB	1330SS	1312sh	1274SB		1248sh	1205SS
													1239SB	
DNP-L-valine 132–133	1541SS	1520SS	1490SS	1466MS	1447sh	1427MS	1406SS	1372SS	1333SB	1299SB			1245SS	
						1425sh	1391sh						1238sh	
													1235sh	
DNP-DL-valine 187–189	1529sh	1520sh	1508SB	1462MS		1412SS		1362MS	1332SB	1309SS	1284SB	1261SB	1242SB	
				1451MS										

TABLE I (*Continued*)

Absorption spectra in the infrared in the 5000–625 cm^{-1} region[a]

DNP-DL-leucine 132–133	1164MS	1148WB	1122MB 1112MB	1095WB		1058WB				958WB			916WS	
Di-DNP-L-lysine 178–180	1181WS	1140MB	1130MB 1107WB			1054WB							917WB	
E-DNP-L-lysine 197–200			1131SB 1122sh 1117SB	1088sh	1076MB	1053MB						922MB		
DNP-DL-methionine 122	1183MS 1170MS	1144SS	1127MS	1100sh 1093MB		1057MB			987WB	966WB 959sh	939WB	921MS	917sh 907WB	
DNP-L-phenylalanine 191	1188SB 1174sh	1156sh 1147SB 1138sh	1130sh	1094sh 1091SB	1071MB	1054SS	1028WB 1026WB	997WB	987WB 976WB	963WB	926MS		913MS	881WB
DNP-DL-phenylalanine 219	1175sh	1159SB 1144SB	1136sh 1131sh	1094SS	1075MS	1054MS	1027WB		983WB	965WB	928WB	923WB 919sh	916sh 909WS	883WB
DNP-L-proline 138	1178MB	1159MS 1145MB	1119MB	1094MB		1063MB	1040WB		976MB				909MB	878WB
DNP-DL-serine 197–201		1153SB	1131sh 1112SB		1067SB	1062sh					934MB		915SB	887MB
DNP-DL-threonine 178–179		1155SB	1126sh 1124sh 1105SB		1083SB	1057SB	1024MB		986WB	966WB	934MB		911MB	
DNP-L-tryptophan 217–221 (*d*)		1143SS	1120MB 1104MB	1093MB		1058MB						923vw	913WB	882WB
Di-DNP-L-tryosine 184 (*d*)	1190MB	1161WS 1144MB	1133sh 1105WB			1064WB 1057sh	1015WB					923MB		889vw
Di-DNP-DL-tryosine 207 (*d*)		1147MB	1121MS 1107MS	1089MB		1062MB		1006WB			939MB 929WB	921vw	914MB	
DNP-L-valine 132–133	1176SS 1174sh 1170sh	1149SS	1125SS 1100SS			1055MS	1029sh			968WS	945WB 926MS			885vwS
DNP-DL-valine 187–189	1174WB	1151SB	1124MB 1100MB			1055WB				953WB		918MB		

TABLE I (*Concluded*)

Absorption spectra in the infrared in the 5000–625 cm^{-1} region[a]

DNP-DL-leucine 132–133		831WS 825WS				763vw	743MS	715WB	709vw	685MB		654WB
Di-DNP-L-lysine 178–180		831WB	817WB			761vw	741MS		709vw 703vw			
E-DNP-L-lysine 197–200		831MS	818MB			763WB	741MS		699MB			
DNP-DL-methionine 122		830MB	817MB	797WB	784WB	762WB	743MS	720WB	714WB	676WB		
DNP-L-phenylalanine 191	849WB	831WB	817SS			768sh 762SS	743MS	717MB	711sh 703SS		661SB	648MB
DNP-DL-phenylalanine 219	855WB	833WB		811MS		765MB	744WB	719MS 717sh	707MS		663sh 662MS	648MB
DNP-L-proline 138		829MS		805MS	773WB	755WS	741SS	724MB		690WB		647WB
DNP-DL-serine 197–201		827SS				768sh 763MB	744SS	715MB		690MB	658MB	
DNP-DL-threonine 178–179	867MB	834sh 828MB				764WB	750MB 744MS	715MB			660WB	
DNP-L-tryptophan 217–221 (*d*)		830MS	821MB			768WB 755MB	741MB		707WB		658WB	
Di-DNP-L-tryosine 184 (*d*)	866vw 855vw	833MB	814vw		784vw	765vw	744MB				667WB	
Di-DNP-DL-tryosine 207 (*d*)		831WB	820MB		778MB	760vw	750MB 742SB		711MB			
DNP-L-valine 132–133		834MS	820SS			762MB	749MB	722SB 718SB				
DNP-DL-valine 187–189		830WB	819MS			761WS	749sh 745MS	720WB		692vw	669WB	

[a]W weak intensity, M medium intensity, S strong intensity, B broad band, S sharp band, sh shoulder, vw very weak band.
[b]These numbers represent uncorrected melting points.

Amino acids have been reported to show marked similarity in their infrared spectra in the region of 1587 to 1333 cm^{-1}. Bands at 1587 and 1408 cm^{-1} have been related, respectively, to the antisymmetrical and symmetrical stretching vibrations of the ionized carboxyl group of the dipolar ions. None of the free amino acids studied lacked the 1408 cm^{-1} band. Among those in which the 1587 cm^{-1} band was not observed were L-threonine and L-proline (3). All of the DNP-derivatives examined by us, however, exhibit the bands at 1587 and 1408 cm^{-1}, and hence the implication is possible that these compounds are dipolar ions. For the free amino acids, a band at 1515 cm^{-1} due to an N–H deformation motion of the α-amino group has been reported, L-leucine, L-serine, and hydroxy-L-proline being among the exceptions (3). Again, there are no such exceptions among the DNP-derivatives studied by us; but we believe that the band here represents antisymmetrical aromatic NO_2 stretch rather than NH-deformation. In the case of free amino acids, bands at 1449 and 1370 cm^{-1} are due to antisymmetrical and symmetrical CH_3 and possibly CH_2 deformation motions, respectively. Among the free amino acids in which the 1449 cm^{-1} band was not apparent were L-valine, L-phenylalanine, hydroxy-L-proline, L-aspartic acid, and L-lysine (3). While the DNP-derivatives of L-aspartic acid and L-lysine (both mono- and di-) and in addition of L-cysteine, L-cystine, L-histidine (di-), L-tyrosine (di-), and DL-threonine do not show the band, those of L- and DL-valine, L- and DL-phenylalanine, and hydroxy-L-proline exhibit it weakly or as a shoulder. In the spectrum of free glycine only, the 1370 cm^{-1} band was lacking (3), and in that of its DNP-derivative it is present. Furthermore, the DNP-derivatives of L-cystine (di-), L-lysine (di-) (unlike the monosubstituted forms), and DNP-L-tyrosine (di-) do not show this band. A 1333 cm^{-1} band has been considered to be related to a CH_2 wagging motion and the only exception among the free amino acids was due to L-alanine (3). None of the DNP-amino acids studied lacks this band. It should be emphasized, however, that bands in the region 1330–1370 will be difficult to interpret, as symmetrical aromatic NO_2 stretch is in this region and is usually intense.

While many free amino acids possessed a band at 2564 cm^{-1}, provisionally assigned to the C–H stretching motion (3), but more likely due to overtone or combinations of the strongly anharmonic $N–H_n$ deformations, it is missing in almost all DNP-derivatives. On the other hand, the spectra of DNP-derivatives with the exception of DNP-L-tyrosine (di-) and DNP-L-arginine possess a band at 1724 cm^{-1}, a band which is not exhibited by the free amino acids.

The spectrum of the racemic form of DNP-alanine differs very little from that of its optically active form. The spectrum of the former does not show the band at 1727 cm^{-1} or that at 1261 cm^{-1}. In the case of DNP-isoleucine, the differences are also not very marked. There is an additional band at 733 cm^{-1} in the spectrum of the active form. The spectrum of the active form of leucine shows additional bands at 1206, 1076, 939, and 775 cm^{-1}. The shoulders at 1420 and at 1203 cm^{-1} of DNP-L-phenylalanine turn into medium broad bands for the DL-form. This L-form has an additional band at 1188, while the DL-form also has an additional band at 1294 cm^{-1}. For tyrosine the shoulder at 3390 cm^{-1} of the L-isomer is a definite dip in the spectrum of the racemic mixture. A striking difference is the absence of the intense and sharp band at 1715 cm^{-1}, the medium band at 1479 cm^{-1}, and the weak one at 1161 cm^{-1} in the DL-form of this amino acid. The DL-form has additional bands at 1362, 1239, 1121, 1089, 939, 750, and 711 cm^{-1}. The shoulder at 1529 cm^{-1} for DNP-DL-valine turns into a definite band for the L-form. The major differences, however, between the spectra for the L- and DL-forms of this amino acid lie between 1429 to 1250 cm^{-1} where the L-form has clearly resolved bands at 1427, 1309, and 1261 cm^{-1}.

The question arises as to whether structurally related DNP-amino acids can be readily differentiated. In the case of DNP-L-proline versus DNP-hydroxy-L-proline, for instance, the former has pronounced additional peaks at 1377, 1290, 1221, 1159, 1094, 1040, and 773 cm^{-1}, the latter at 1245, 997, and 927 cm^{-1}. The 3226-cm^{-1} band, however, reported to be present in the free hydroxy-prolines but not in the spectrum of free proline and assumed to reflect the presence of the —OH group in the former (3), is absent in the spectra of the DNP-derivatives of either L-hydroxyproline or L-proline.

The spectra of DNP-L-valine and DNP-L-isoleucine would be expected to resemble each other. DNP-L-isoleucine has additional bands at 3058, 1222, 1121, and 733 cm^{-1}, DNP-L-valine at 1490, 1427, 1176, and 945 cm^{-1}. In the case of the spectrum of the corresponding free amino acids, L-isoleucine, unlike L-valine, shows a sharp and discrete band at 1462 cm^{-1} (3). Such a distinguishing difference is ruled out for the DNP-derivative of these amino acids.

It would be surprising if the infrared spectra of closely related compounds did not reveal some differences by which pure, individual samples of such compounds could be differentiated from each other. A review of the table suggests that among the DNP-L-amino acids studied the spectra on the whole are more strikingly similar than they are different, and that the use of the spectral tool for purposes of identification is much weaker and less reliable than such procedures as chromatography. There are, however, some DNP-amino acids (e.g., leucine and isoleucine) which are difficult to identify by chromatography and hence spectral analysis after purification would still be of value. Even the spectra of structurally very similar DNP-amino acids show sufficient variation to allow positive identification.[6]

REFERENCES

1. SANGER, F. Biochem. J. **39**, 507 (1945); **45**, 563 (1949).
2. LEIFER, A. and LIPPINCOTT, E. R. J. Am. Chem. Soc. **79**, 5098 (1957).
3. KOEGEL, R. J., GREENSTEIN, J. P., WINITZ, M., BIRNBAUM, S. M., and MCCALLUM, R. A. J. Am. Chem. Soc. **77**, 5708 (1955).
4. RAMACHANDRAN, L. K., EPP, A., and MCCONNELL, W. B. Anal. Chem. **27**, 1734 (1955).
5. LEVY, A. L. and CHUNG, D. J. Am. Chem. Soc. **77**, 2899 (1955).
6. RAO, K. R. and SOBER, H. A. J. Am. Chem. Soc. **76**, 1328 (1954).

[6]*While it is recognized that polymorphism can result in spectral changes due to the alterations in the immediate environments of the vibrating groups, no attempt has been made here to evaluate this factor.*