Infrared spectra of amino acid and peptide monoderivatives of [60]fullerene and their methyl esters

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IR spectra (4000–400 cm^{-1}) of [60]fullerene monoderivatives of amino acids and dipeptides and several of their methyl esters have been found to differ strongly from those of starting compounds, in that none of them contained the characteristic modes of free C_{60} but instead displayed several new absorptions which indicate the presence of amino acid and peptide moieties in the [60]fullerene derivative molecules, these fragments being in a zwitterion form in most cases.

In recent years the vibrational spectra of [60]fullerene have been studied in detail whereas less attention has been paid to those of fullerene derivatives. Especially, little is known about the spectra of fullerene monoderivatives. They are less available since multiaddition generally occurs in the addition reactions of fullerenes.

In the present paper, we report the IR spectra of a series of amino acid and dipeptide derivatives of C_{60}, $HC_{60}NHXCOOH$ and $HC_{60}NXCOOH$, formed on addition of one primary or secondary amino acid or dipeptide molecule to one of the fullerene core double bonds.[1] Following addition, several derivatives of the carboxyl group of the compounds under question were obtained. This indicates that the addition process involves the amino group whereas the carboxylic group remains unaltered.[†] All peptide derivatives of fullerene and some of the amino acid derivatives are soluble in water, where they form micelle-like aggregates.[2] However, at small concentrations they are capable of giving true solutions. In such cases the molecular masses of compounds calculated from diffusion experiments were shown to correspond strictly to those of monoderivatives.[3]

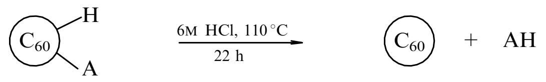
In the course of electrophoresis, water soluble amino acid or dipeptide derivatives of fullerene in acetate–pyridine solution moved towards a positively charged electrode as single spots. This indicated the presence of individual compounds rather than mixtures.[4] We failed to observe the molecular ions of these compounds in their mass spectra. However, upon acidification the mass spectrum displayed the sharp peaks of the isotopically distributed radical cation of fullerene. To obtain additional data on the structure of amino acid/dipeptide derivatives of fullerene we carried out an amino acid analysis of the compounds synthesized. The 1:1 ratio of amino acid to fullerene provides the final proof that only one molecule of the amino acid adds to fullerene.[4]

The synthesis of amino acid and peptide derivatives of [60]fullerene has been described by us earlier.[1]

The composition of the compounds prepared was studied by amino acid analysis after destruction by heating in the presence of 6M HCl. The results of the analysis (Table 1) show that all compounds contain only one amino acid/peptide residue and therefore they are monoderivatives of fullerene.

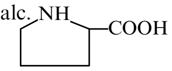
The C_{60} molecule possesses the symmetry of the icosahedron. Due to this high degree of symmetry, most of the 174 vibrational modes of C_{60} are degenerate and this reduces the number of observed vibrational frequencies to 46. Of these, only four belonging to the F_{1u} class are active in the IR spectrum (1429, 1183, 577 and 528 cm^{-1}); ten are active in the Raman spectra: 1469, 497 (A_g class), 1572, 1426, 1248, 1099, 773, 708, 431 and 270 cm^{-1} (H_g class). The C_{60}^- ion exhibits the same symmetry and number of vibrational modes. However, its frequencies are shifted with respect to the neutral molecule, revealing themselves in the IR spectrum as bands at 1340, 1185, 564 and 546 cm^{-1}. Addition of an organic group to the fullerene core reduces the symmetry of the molecule and, generally speaking, should result in an increase in the number of bands observed. Formally, addition to one of the fullerene multiple bonds reduces the symmetry from I_h to C_s and should remove the degeneracy of vibrations and allow all 174 modes to be seen in the IR spectrum. In reality, such complication is not observed in the spectra of fullerene derivatives. The addition of one group may not influence the mechanics of vibrations of such a large molecule as fullerene to a great extent, and the derivative will continue to behave as a quasi-icosahedron retaining the degeneracy for most vibrations. Similar behaviour has been observed for the icosahedral molecule carborane(12)[5] although it is smaller than fullerene and so the influence of substituents should be greater. Apparently, the vibrational modes of fullerene can be considered as a sum of interacting vibrations of the six- and five-membered rings constituting the molecule, most of them changing insignificantly upon addition of an organic group. However, the appearance of new bands relating to the fullerene fragment attached to the substituent, characteristic of fullerene-added group modes, and to the internal vibrations of the added group can be anticipated. Also, provided the added group strongly affects the distribution of the electronic density in the C_{60} molecule, the intensities of some bands can

Table 1 Amino acid analysis data obtained from the hydrolysis of amino acid and peptide derivatives of fullerene [60].



A = NHCHRCOOH;
NHCHRCONHCHR′COOH

AH = $NH_2CHRCOOH$;
$NH_2CHRCOOH$ +
$NH_2CHR'COOH$

Amino acid/peptide, added to C_{60}	Amount of amino acid in the sample (μmol) Found	Calc.	Found : Calc.
			
NH_2CH_2COOH	4.20	4.40	0.96
$NH_2CHMeCOOH$	1.36	1.34	1.02
$NH_2CH(CH_2OH)COOH$	1.14	1.27	

[†] This also follows from the IR data reported in this communication.

Table 2 Characteristic frequencies (cm^{-1}) in the IR spectra of C_{60} aminoacid and peptide monoderivatives (in KBr pellets).

Aminoacid/ peptide/ester	$\nu_{NH} + \nu_{OH}$	ν_{COO^-}	ν_{COOH}	Ester and amide bands	New bands	C_{60} ?	Note
Glycine	3440 s,br 3230 w,br	1589 s 1404 s			1249 w,1106 s, 965 m, 837 m	527 vw	zwitterion
Alanine	3407 m,br 3280 sh	1592 s 1407 m	1681 m		1252 w, 1108 vs 962 s, 837 m	1176 w, 528 w	zwitterion + acid
Serine	3406 vs,br 3260 sh	1597 s 1394 s	1721 m		1251 w, 1106 vs 960 s, 837 m	530 vw	zwitterion + acid
Aminocaproic acid	3438 s.br		1705 m		1104 m, 844 w	528 w	acid
	3371 m.br[a]	1560 s 1413 s	1736 m		1107 m, 934 w		zwitterion + acid
Aminocaproic acid methyl ester	3372 vw			1733 m	1244 w, 1109 vs, 960 s, 837 m	525 vw	
p-Aminobenzoic acid	3420 w. br		1693 s		1257 w, 1105 m 960 w, 841 w	1419 m, 1173 m, 574 w, 527 w	acid
Proline	3300 s.br		1716 m		1250 vw, 1107 s, 961 m, 837 m		acid
Proline methyl ester		1606 m 1350 s		1742 w	1247 s, 1101 vs, 965 vs, 837 s	528 m	
Glycylglycine	3420 vs,br 3278 sh	1610 s 1352 s		1653 s	1250 w, 1106 vw, 965 s, 837 m	528 vw	zwitterion
Glycylalanine	3414 vs,br 3279 sh	1604 s 1353 s		1659 s	1251 m, 1106 vs 962 s, 837 m	527 vw	zwitterion
Glycylvaline	3420 vs,br 3260 sh	1598 vs 1398 s		1672 vs	1252 m,1108 vs 962 s, 836 m	576 vw	zwitterion
Alanylalanine	3405 vs,br 3267 m, br	1597 vs 1352 s		1654 vs	1251w, 1107 vs, 960 m, 837 m		zwitterion

[a] Solid film.

change substantially, in particular the bands forbidden with respect to symmetry can appear.

In most of the addition reactions described in the literature several molecules (from 6 to 24) are added to fullerene. Few examples have hitherto been reported in which monoaddition occurred. Almost all C_{60} bands have been found to remain unchanged in the IR spectrum of $C_{60}Pt(PPh_3)_2$.[6] Also, fullerene bands were reported for the fullerene–anthracene adduct (1:1), whereas those characteristic of anthracene were strongly changed.[7] The spectra of hydrofullerenyl amines have been reported. In the case of secondary amines, the IR spectra retained the fullerene bands, lacked the NH band of amine, and displayed a new, very weak one at 3040 cm^{-1} which shifted to 2240 cm^{-1} upon deuteration. This band was ascribed to the vibrational mode of a new C–H bond.[8] Subsequently, these data were revised: the 3040 cm^{-1} band was assigned to the hydrogen bond with water, and the band at 2240 cm^{-1} was not observed.[9] The presence of fullerene bands was also reported for hydrofullerenyl derivatives of butylamine, ethylene-

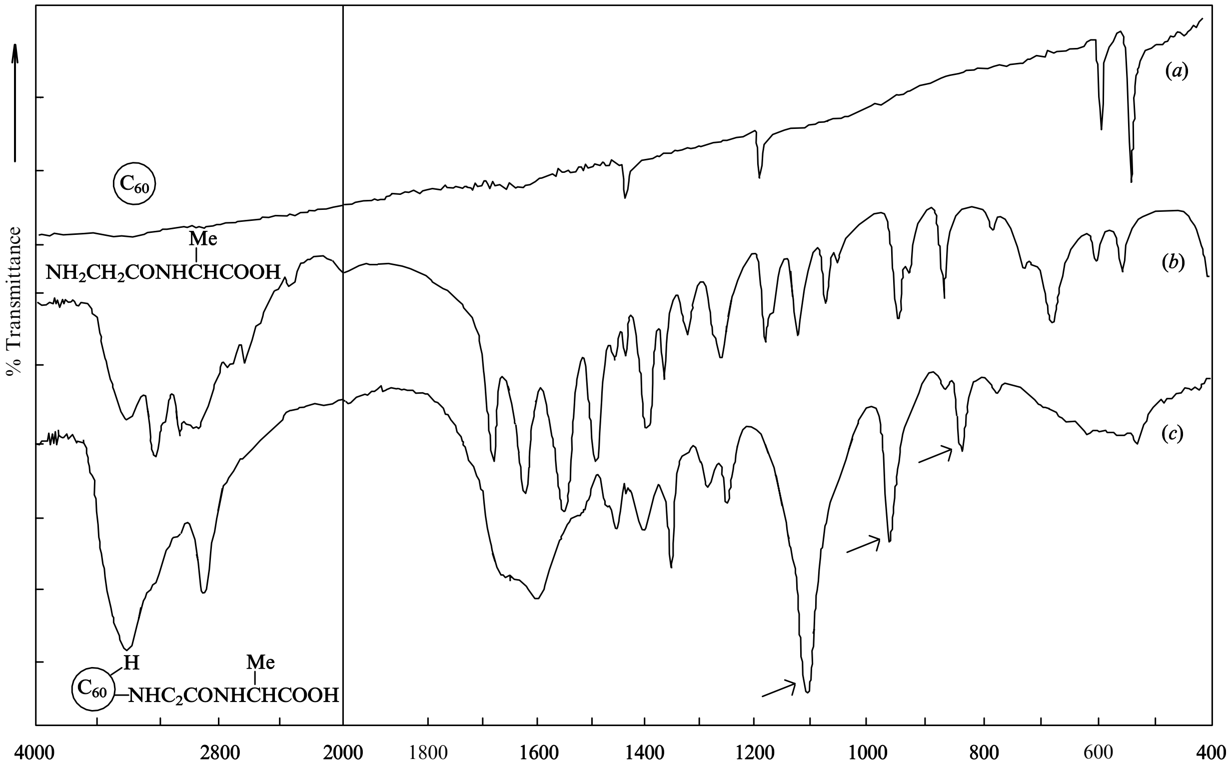


Figure 1 IR spectra of (*a*) C_{60}; (*b*) glycylalanine; and (*c*) compound **8**.

diamine[10] and azacrownethers.[11] However, none of the C_{60} bands were observed in the IR spectra of hydrofullerenyl derivatives of some diamines,[12] but new bands (1500, 1416, 1100, 1080 and 960 cm^{-1}) appeared in the IR spectrum of the hydrazine derivative[10] and were assigned as the changed modes of C_{60}. Thus, the data on the spectra of monoaddition products that has been presented in the literature so far are very poor and contradictory. Also, all the reported IR data have been in papers dealing with the synthesis of derivatives and contains only selective frequency values, lacking a complete description of the IR spectra.

The present work reports an IR spectral study[‡] of the reaction products of C_{60} with a series of amino acids (glycine **1**, alanine **2**, serine **3**, proline **4**, ω-aminocaproic **5** and *p*-amino-benzoic **6**) and also with dipeptides (glycylglycine **7**, glycyl-alanine **8**, glycylvaline **9** and alanylalanine **10**). The methyl esters of compounds **2**, **4** and **5** have been also studied.

A marked difference between the spectra of C_{60} and of amino acids and those of the derivatives was immediately revealed. This is exemplified by Figure 1, which shows the IR spectra of glycylalanine and its C_{60} derivative **8**. The additivity of spectral characteristics is lacking. As for C_{60}, amino acids and dipeptides are characterized by their narrow band spectra while the spectra of the derivatives generally contain broader and fewer bands. This indicates that the interaction is accompanied by substantial rearrangement of the electronic srtucture of both components, leading to a strong change both in mechanical and electrooptical properties. Table 2 shows the positions of some of the characteristic bands pertinent to the discussion of the structure of the compounds studied.

We first consider the modes that are characteristic of amino acids and peptides. The strong absorption bands in the regions 1590–1620 cm^{-1} and 1350–1420 cm^{-1} are observed for all compounds except **4**, **5** and **6**. These bands are characteristic of asymetric and symmetric stretching modes of the carboxylate anion COO^- (ν^{as} COO^- and ν^{s} COO^-). This finding both indicates the presence of an amino acid moiety in the derivative and shows that the derivatives exist as zwitterions, as is the case with the starting amino acids. The spectrum of compound **5** recorded in a KBr pellet contains a band at 1705 cm^{-1} characteristic of the carboxylic group while a spectrum obtained in the film deposited from a pyridine solution of **5** displays two bands at 1603 and 1413 cm^{-1} that are characteristic of the ionized group COO^-. The spectrum of a film of compound **4** contains a strong band at 1716 cm^{-1} which is characteristic of the COOH group (this derivative is the only representative of secondary amino acids explored by us). However, some difficulties have arisen in assigning the bands in the 1600–1700 cm^{-1} region since the deformation modes of NH groups should occur in this region as should those of water molecules retained in the samples by means of hydrogen bonds. The presence of a dipeptide moiety is evidenced by the existence of strong absorption bands in the region 1650–1670 cm^{-1} (the 'Amide **1**' band) and one of medium intensity at *ca.* 1520 cm^{-1}. The spectra of all methyl esters studied display the bands in the 1730–1745 cm^{-1} region that belong to the ester group.

The spectra of all derivatives explored contain broad absorption bands with maxima at *ca.* 3400 cm^{-1}, occasionally possessing shoulders at 3200 cm^{-1}, which can be ascribed to the N–H stretching modes of the zwitterionic ammonium groups or to the amine groups of the amide forms. In the latter case, these bands may represent the superposition of the above absorptions with ones of the carboxylic 'OH' and, also, of the side-chain 'OH' group in the case of the serine derivative.

The presence of fullerene molecule vibrations in the complex is of special interest. A very weak, narrow band has been observed in the IR spectra of the derivatives at *ca.* 530 cm^{-1}, just within the region of the most strong C_{60} band; this band is, sometimes, noticable against the background of other, broader bands. In the spectra of some derivatives there exist very weak bands at *ca.* 580, 1180 and 1420 cm^{-1}. These bands are rather intense in the spectrum of C_{60}. Our measurements of the molar extinction coefficient (ε) in a benzene solution gave values of 1530 and 530 for the 527 and 578 cm^{-1} bands respectively. This leads us to dismiss the suggestion that the weak fullerene bands almost melt into the background of the strong bands of the derivative.

A characteristic feature of the spectra appears to be the existence of a specific group of three bands at *ca.* 1108 cm^{-1} (s), 960 and 840 (m), and a very weak one at 1250 cm^{-1}. These bands are distinctly developed for all compounds except compound **5** where they are probably masked by other broad absorption bands. Interestingly, their position and relative intensities are virtually independent of the structure of the derivative. The bands appear for both ionic and acidic forms and also for esters and dipeptide derivatives. The assignment of these bands is not clear; they probably relate to vibrations of the fullerene at the point of substitution. One of these bands may correspond to the streching modes of the C_{60}–N bond or to the deformation mode of the C_{60}–H bond. It is noteworthy that these new bands have also been observed at *ca.* 1100 and 960 cm^{-1} for hydrofullerenylhydrazine.[10]

Another unsolved problem seems to be the position of a band corresponding to the C_{60}–H stretching mode. As mentioned above, the assignment of the 3040 cm^{-1} band to this vibration[8] has been doubted.[9] Comparison of the spectra of the compounds studied with those of starting amino acids and their derivatives has demonstrated that upon addition to C_{60} the contour of the band related to the C–H stretching modes changes, and a new band appears which shifts the maximum of this contour to 2900–2920 cm^{-1}. Since the addition of a hydrogen atom to C_{60} changes the sp^2 hybridization of the corresponding carbon atom to sp^3, the assignment of this new band, which appears in the region of aliphatic C–H bond vibrations, to $\nu_{C_{60}\text{–H}}$ seems reasonable.

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[‡] IR spectra were taken on a FT Bruker IFS-113V spectrometer in the 4000–400 cm^{-1} region. The samples were prepared in the form of the KBr pellets, Nujol mulls or solid films deposited from solutions onto the surface of the KRS-5 window.