A Spectroscopic Investigation of Losartan Polymorphs

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Losartan, an antihypertensive agent in clinical development, was found to exist in two enantiotropic polymorphic forms, a low-temperature stable form (Form I) and a high-temperature stable form (Form II), the temperatures at which they are stable being related to the transition temperature. X-ray powder diffraction patterns indicated differences in the crystal packing of the two forms. The vibrational data from infrared and Raman spectroscopy suggested a subtle change in molecular conformation and crystal packing in the two forms. Solid-state ^{13}C NMR data of the polymorphs concurred with the vibrational data and indicated that, while the observed line widths reflect no major changes in crystallinity, signal multiplicities and chemical shifts do reflect differences in molecular packing in the respective unit cells. Thus, in the absence of crystallographic data, useful structural information could be derived from spectroscopic results to identify each of the crystalline forms.

KEY WORDS: polymorphs; infrared; Raman; solid-state nuclear magnetic resonance; crystal packing.

INTRODUCTION

The renin angiotensin system is a target of the antihypertensive ACE inhibitors captopril and enalpril. However, serious side effects associated with ACE inhibitors prompted an intensive effort aimed at discovering drugs with a more specific mechanism of action and the result was the discovery of Losartan, the first orally active nonpeptide AII receptor antagonist currently being evaluated in the clinic for the treatment of hypertension.

Losartan (Fig. 1) was found to have at least two polymorphic forms. The current work is aimed at characterizing the polymorphs based on thermal and solubility data and analyzing their solid-state characteristics by nuclear magnetic resonance (NMR), infrared (IR), and Raman spectroscopy in relation to their crystal packing.

Infrared and Raman spectroscopies have been widely used to elucidate molecular structures, crystallinity, and polymorphism. The low-frequency Raman modes are particularly useful in distinguishing different molecular packings in crystals (1). Solid-state ^{13}C NMR spectroscopy is also used in the characterization of pharmaceutical compounds (2). ^{13}C spectra acquired using a combination of cross polarization (CP) (3) for sensitivity enhancement with magic-angle spinning (MAS) (4) and high-power proton decoupling for resolution enhancement have been shown to provide relevant structural and dynamic information (5). Although the attainable resolution typically falls short of that available from the corresponding experiment in solution, solid-state NMR spectroscopy has the decided advantage that observed chemical shifts are influenced by molecular packing in the crystal lattice. This makes ^{13}C CP/MAS NMR spectroscopy particularly well suited for investigating polymorphism in pharmaceuticals. Also, since the experiment is usually performed on powdered solids, structural information may be deduced on these materials in their appropriate dosage forms.

MATERIALS AND METHODS

Losartan (Form I) was prepared by the Chemical Processing Division of The Du Pont Merck Pharmaceutical Company. Polymorph Form II was made by heating Form I in a differential scanning calorimetric (DSC) cell in an open pan to 255°C at a heating rate of 10°C/min. The thermal properties of the polymorphs were characterized on a DSC Model 910 (Du Pont Instruments) with data analysis on a thermal analyzer Model 1090 (Du Pont Instruments).

Fourier transform infrared (FTIR) spectra of the two polymorphs were acquired on an Analect AQS-20 spectrometer equipped with a nitrogen-cooled MCT detector. The samples were finely ground with potassium bromide and the spectra were recorded at 4-cm^{-1} resolution using a diffused reflectance accessory.

Raman spectra were recorded on a Spex 1877 triple spectrometer equipped with a photomultiplier tube and optical multichannel detectors. The samples placed in quartz capillary tubes were excited by a 514.5-nm beam from a Coherent Innova-70 argon ion laser. The laser power at the samples was adjusted to about 150 mW and the spectral resolution was about 4 cm^{-1}.

No attempt was made to assign all the observed modes in the IR and the Raman spectra. Spectral regions where significant differences were observed for the two polymorphs were assigned based on published literature on related compounds (6).

Solid-state ^{13}C nuclear magnetic resonance spectra were acquired on a Chemagnetics CMX-360 NMR spectrometer operating at 90.5 MHz for ^{13}C and 360 MHz for ^{1}H using the CP/MAS technique. Approximately 200 mg of each polymorph was used in the acquisition of their respective spectra. All measurements were made at ambient temperature. Chemical shifts are reported on the TMS scale using hexamethylbenzene as a secondary reference. Solid-state resonance assignments were made using the interrupted decoupling pulse sequence in combination with solution-state ^{13}C experiments performed on a GE Omega-500 high-resolution NMR spectrometer.

A positive assignment of the origin of signal multiplicities in the spectra required additional ^{13}C CP/MAS NMR experiments to be performed at a lower static field strength.

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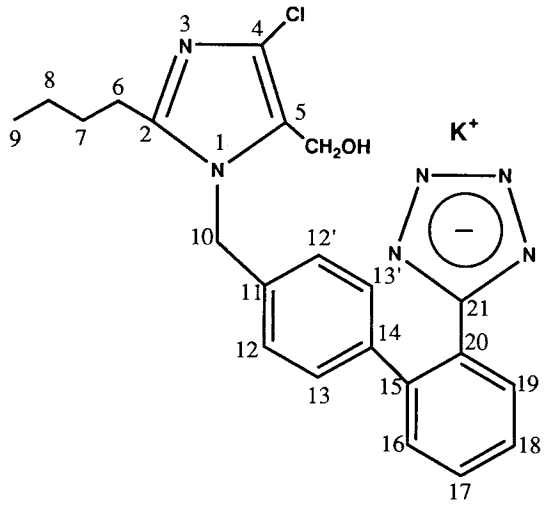

Fig. 1. Losartan.

This was done on a 100-MHz spectrometer with a ^{13}C resonance frequency of 25.2 MHz.

X-ray powder diffraction (XRPD) patterns were recorded using an automated X-ray diffractometer APD 3720 with copper tube K alpha radiation.

RESULTS AND DISCUSSION

The DSC curve for Losartan (Fig. 2A) shows a minor endotherm at an extrapolated onset temperature of 229.5°C and a major melting endotherm at an extrapolated onset temperature of 273.2°C. The minor endotherm disappeared from samples which were heated to 255°C (Fig. 2B). While HPLC, solution ^1H NMR and visual inspection of the sample heated past the minor endotherm did not indicate any chemical change, the XRPD pattern (Figs. 3A and B) indicated a change in the crystal structure. From these facts it was concluded that the minor endotherm corresponds to an enantiotropic polymorphic transition. The major endotherm is the melting of the high-temperature form. The low-temperature stable polymorph (up to the transition temperature) is designated Form I and the high-temperature stable polymorph is designated Form II. Solubility studies with Forms I and II (prepared from DSC) in nonaqueous solvents indicated that Form II converts to Form I after overnight equilibration at 25°C in isopropanol (~35 mg/g), methyl ethyl ketone (~1 mg/g), and ethyl acetate (~0.3 mg/g). In isopropyl acetate at 25°C after overnight equilibration, no conversion of either Form was observed and the solubilities for Forms I and II were 18 and 41 μg/mL, respectively. The solubility studies at 25°C confirm the conclusion that Form I is the more thermodynamically stable polymorph at room temperature. Form I is the solid modification consistently obtained by solvent isolation. This was confirmed by recrystallizing the drug substance under varying conditions and analyzing by XRPD. Form II has been obtained only from DSC or related high temperature experiments.

FTIR spectra of the two polymorphs in the region of 1800–600 cm^{-1} are shown in Figs. 4 and 5. While many of the spectral features are similar, there are discernible differences. Form I shows a greater multiplicity in the spectral region of 700–850 cm^{-1} (Fig. 4A), where the modes are due primarily to C–H out-of-plane bending vibrations in the ar-

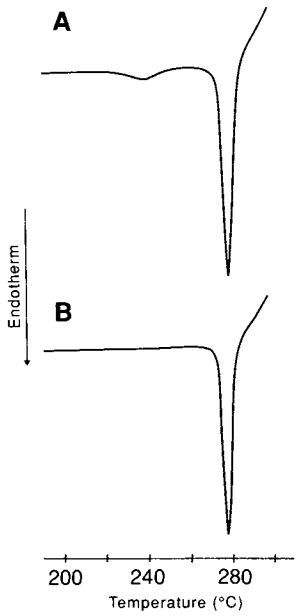

Fig. 2. DSC thermograms of Losartan (A) before and (B) after heat treatment at 255°C. The heating rate was 10°C/min. The material before heat treatment is identified as Form I and the heat-treated material as Form II in the text.

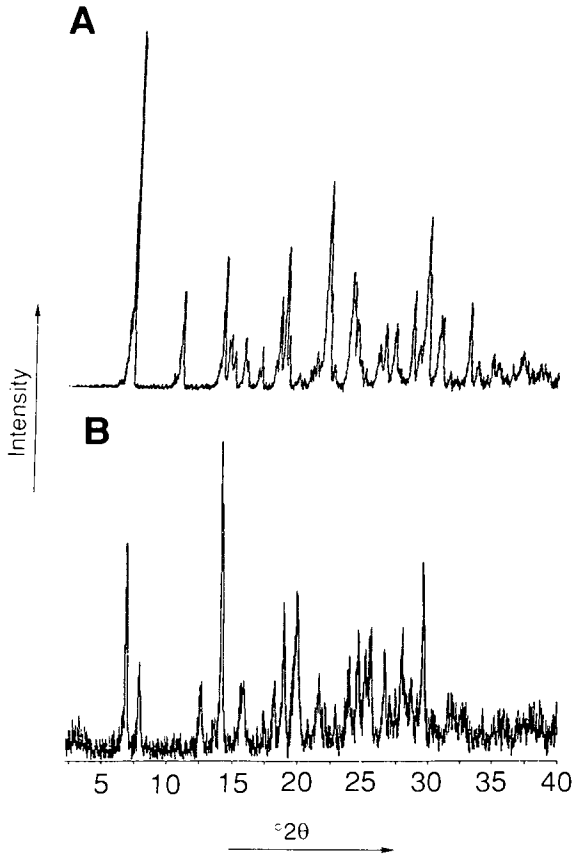

Fig. 3. X-ray powder diffraction pattern of Losartan polymorphs: (A) Form I and (B) Form II.

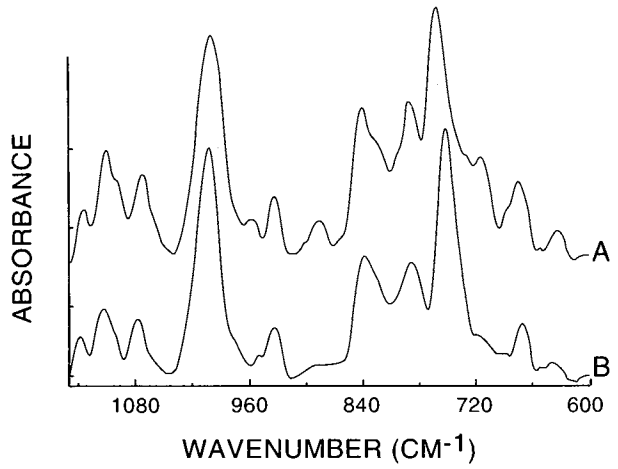

Fig. 4. FTIR spectra of Losartan polymorphs from 1150 to 600 cm^{-1}: (A) Form I and (B) Form II.

omatic rings (6). In this region Form I has four modes, compared to only three modes for Form II (Fig. 4B). We believe that the additional mode in Form I arises due to splitting of the C–H out-of-plane bending mode which occurs around 750 cm^{-1} in the absence of any splitting. Presumably, the molecular packing in this polymorph is such that the aromatic rings from two different molecules are so oriented allowing Van der Waals interactions and an interaction between the transition dipole moments associated with the C–H out-of-plane bending modes. Such interactions are known to cause vibrational splitting. Thus, there are two modes, at 764 and 713 cm^{-1}, in Form I. In Form II, the molecules are arranged differently, not allowing a similar intermolecular interaction, and only one band around 754 cm^{-1} is observed. The imidazole ring modes which occur in the region of 850–970 cm^{-1} (6) show three absorption bands, at 886, 934, and 953 cm^{-1}, in Form I, compared to only one absorbance around 940 cm^{-1} in Form II (Fig. 4). In the aliphatic region, a single mode around 1357 cm^{-1} in Form II, attributed to the C–H symmetrical bending vibration in the methyl group of the *n*-butyl chain on the imidazole ring (6), is observed to be split in Form I (Fig. 5). The IR data suggest that the differences in absorption pattern between the two polymorphs are due to differences in intermolecular interactions in the two crystal forms.

The Raman spectra of the two polymorphs in the spectral region of 600–1100 cm^{-1} are shown in Figs. 6A and B. Although Raman and infrared techniques compliment each other, they have different symmetry dependent selection rules. The C–H out-of-plane motion in the biphenyl ring, for example, is intense in the IR (around 754 cm^{-1} in Fig. 4B), while it is very weak in the Raman and is observed around 763 cm^{-1} (6) in the Raman spectra of Form II (Fig. 6B). This band, however, is split in Form I and the two modes appear at 710 and 760 cm^{-1} (Fig. 6A). A ring breathing mode associated with the imidazole ring (6) observed at 803 cm^{-1} in the Raman spectra of Form II (Fig. 6B) is split in Form I, appearing at 807 and 819 cm^{-1} (Fig. 6A).

The low-frequency Raman spectroscopy provides valuable information, as the Raman modes in this region arise largely due to lattice vibrations that are very sensitive to structural changes in the solid state (1). The Raman modes in this region are somewhat difficult to assign because of their mixed nature. In this spectral region (Fig. 7), Form II has one band around 191 cm^{-1}, where there are two modes, at 199 and 227 cm^{-1}, in Form I. The Raman data suggest that the differences in the spectral pattern between the two polymorphs are due to differences in intermolecular interactions and differences in crystal symmetry in the two forms.

The solid-state ^{13}C CP/MAS NMR spectra of the two polymorphs of Losartan are presented in Figs. 8 (upfield region) and 9 (downfield region). Table I lists the chemical shifts for the two solid polymorphs of Losartan and compares them to the corresponding ^{13}C solution-state values. Exact assignment of solid-state resonances between about 125 and 135 ppm is somewhat difficult due to the high degree of spectral overlap in this region.

The aliphatic ^{13}C spectral region for Form I (Fig. 8A) contains more resonances than are found in the corresponding regions of Form II (Fig. 8B) or in solution (Table I). Peaks at 14.5 and 17.2 ppm survive interrupted decoupling, implying that these are both methyl carbon signals. Since Losartan contains only one methyl group per formula unit,

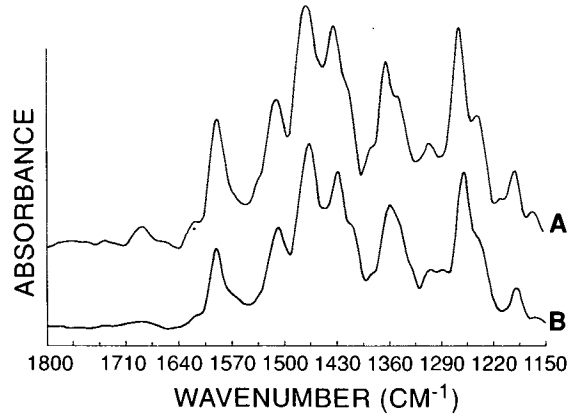

Fig. 5. FTIR spectra of Losartan polymorphs from 1800 to 1150 cm^{-1}: (A) Form I and (B) Form II.

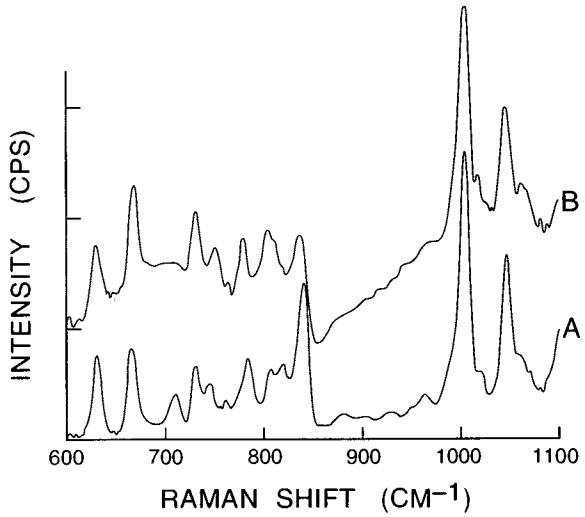

Fig. 6. Raman spectra of Losartan polymorphs from 1100 to 600 cm^{-1}: (A) Form I and (B) Form II.

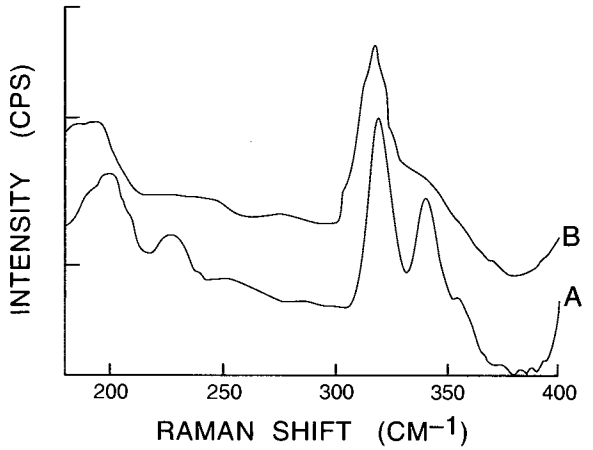

Fig. 7. Raman spectra of Losartan polymorphs from 180 to 400 cm^{-1}: (A) Form I and (B) Form II.

this suggests the presence of more than one orientation for the *n*-butyl side chain in the unit cell.

The analysis may be taken one step further by considering the relative peak areas for the two methyl signals in Fig. 8A. The observed unequal integrals of the methyl signals at 14.5 and 17.2 ppm suggest a corresponding unequal distribution of aliphatic chain conformations in the unit cell. On closer inspection, one can see similar multiplicity patterns in other resonances in Fig. 8A. Methylene carbon C8 (21.2 ppm), for example, may also account for the small signal near 25 ppm. Aliphatic signal multiplicity is actually more readily observed in the 25-MHz ^{13}C spectrum (not shown), where it appears that at least three of the signals (C7, C8, C9) may be similarly split.

The downfield ^{13}C spectral region of Form I is shown in Fig. 9A, and signal assignments are given in Table I. Note that peak C2 of the imidazole ring is split into a doublet. While the pattern is rather similar to the chemical shift splitting observed for this polymorph in Fig. 8A, it is also much like that typically observed in CP/MAS spectra for ^{13}C's

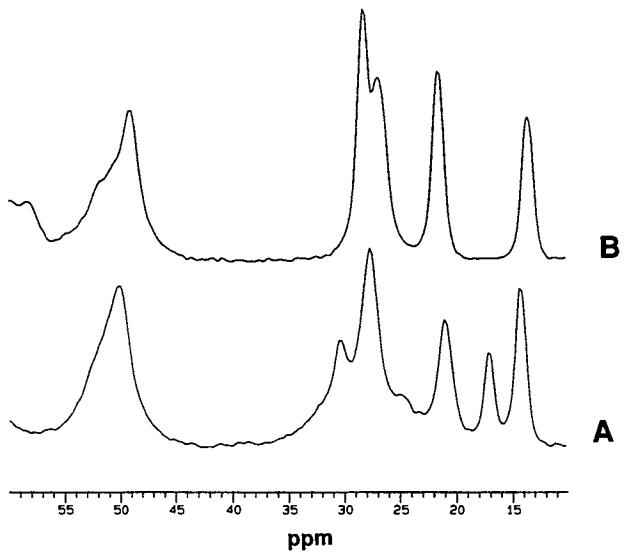

Fig. 8. Solid-state ^{13}C CP/MAS NMR spectra (upfield region) of Losartan polymorphs: (A) Form I and (B) Form II.

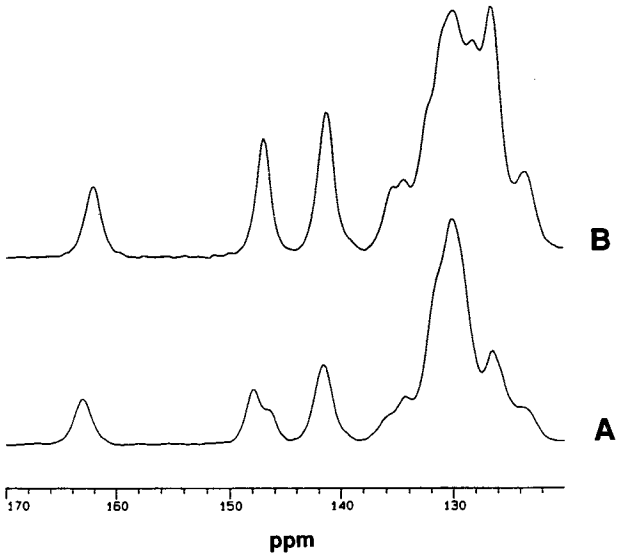

Fig. 9. Solid-state ^{13}C CP/MAS NMR spectra (downfield region) of Losartan polymorphs: (A) Form I and (B) Form II.

attached to nitrogen (7). MAS is unable to remove completely coupling to quadrupolar nuclei like ^{14}N, and residual broadening is often manifest as line splitting in ^{13}C spectra. This apparent ambiguity about the origin of the C2 line shape may be sorted out by exploiting the different field dependence of the two effects (8) and comparing CP/MAS spectra obtained, in this case, at 90 and 25 MHz. Line shapes are not consistent with residual coupling of C2 to nitrogen but, rather,

Table I. Solution ^{13}C NMR Chemical Shifts for Losartan in Deuterated Dimethyl Sulfoxide and ^{13}C CP/MAS Solid-State NMR Chemical Shifts for Form I and Form II[a]

Carbon no.	^{13}C chemical shifts [ppm]		
	Solution (DMSO-d_6)	Solid state	
		Form I	Form II
21	160.6	163.2	162.2
2	147.3	148.1, 146.5	147.1
15	141.1	141.6	141.5
14	139.8	141.6	141.5
11	134.5	136.2, 134.7	135.6, 134.5
5	132.5	131.9	132.6
19	130.4	130.2	130.2
16	129.9	130.2	130.2
13, 13′	129.3	130.2	130.2
17	127.2	130.2	128.4
18	126.6	130.2	128.4
20	125.5	126.5	126.7
4	125.3	123.4	123.6
12, 12′	125.2	130.2	126.7
CH_2OH	51.3	50.4	52.0
10	46.5	50.4	49.1
7	29.0	30.6	28.3
6	25.8	27.9	26.8
8	21.6	21.2	21.5
9	13.6	17.2, 14.5	13.7

[a] All chemical shifts are relative to tetramethylsilane, and numbered carbon atoms refer to the structure in Fig. 1.

indicate that the signals between 146 and 148 ppm constitute a chemical shift multiplet. The structural significance of this result then parallels that discussed for Fig. 8A above.

No such peak splitting is observed for the corresponding spectral regions of Form II (Figs. 8B and 9B). Heating therefore appears to change the packing in the crystal in such a way that the unit cell of this form contains only one crystallographically unique molecule. Other, more subtle, differences between spectra in Figs. 8 and 9 are also indicative of packing differences between the two polymorphs under investigation. Some resonances are shifted (Table I), and spectral intensity is clearly distributed differently in the aromatic region between about 120 and 136 ppm. It is important to note that, while heating appears to change the solid-state packing in Losartan, similar ^{13}C line widths for the two polymorphs indicate that there is no net change in the crystallinity of the sample, concurring with the XRPD data.

A final observation regarding the high-temperature polymorph of this drug concerns dynamics of the aliphatic chain. Three of the four aliphatic resonances in Fig. 8B survive in the interrupted decoupling experiment. There is apparently enough molecular motion associated with carbons C7, C8 and C9 to render the corresponding ^{13}C–^{1}H dipolar couplings weaker than those found in most rigid, crystalline solids (including Form I). This added degree of molecular motion seems consistent with a more loosely packed crystal structure for Form II.

In summary, Losartan was found to exist in two enantiotropic polymorphs, a low-temperature stable Form I and a high-temperature stable Form II. DSC was used along with XRPD to establish that the two forms were enantiotropically related. Solubility studies confirmed this and Form I was shown to be the most stable form at room temperature. The FTIR and Raman spectra of the two crystal forms were very similar with minor but discernible differences. The solid-state ^{13}C NMR spectra of the two forms revealed marked differences in the chemical shift and peak splitting characteristics. The spectral characteristics of Form I were interpreted in terms of the presence of more than one orientation for the *n*-butyl side chain and the imidazole ring. In addition, the spectral characteristics of Form II were consistent with a large molecular motion of the *n*-butyl side chain.

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