The activity was eluted as two separated peaks, of which the minor peak corresponded to less than 5 % of the total yield of enzyme activity.

The major peak of activity was collected, concentrated by ultrafiltration as above, and analyzed for homogeneity in polyacrylamide gel electrophoresis.[2] In order to localize the glycyl-L-leucine dipeptidase activity in the polyacrylamide gel and relate it to the protein, the gel was cut longitudinally into two halves. One half was stained with Coomassie Blue and the other was cut in 1 mm thick slices, which were separately extracted in 0.02 M Tris-HCl (pH 7.3), and assayed for enzyme activity. The result of a typical experiment is shown in Fig. 2. Repeated gel electrophoresis of the major band after concentration showed the same distribution of protein into three bands. This result indicates a transformation between different forms of the enzyme occurring during the experiment, a phenomenon known from other polyacrylamide gel electrophoresis experiments.[3]

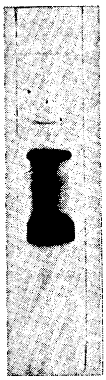

Fig. 2. Polyacrylamide gel electrophoresis (pH 9.3) of glycyl-L-leucine dipeptidase. 100 μg of the major peak of a Sephadex G-100 chromatography were applied. Most activity was found in relation to the strongest band but the weaker bands also corresponded to traces of activity. The gel was stained with 0.04 % Coomassie Brilliant Blue in 10 % trichloroacetic acid. 2.5 mA was applied for 90 min.

Each purification usually starts with about 90 g of lyophilized mucosa extract and gives about 2 mg of the purified glycyl-L-leucine dipeptidase (major peak of Sephadex G-100 effluent, Fraction 6, Table 1).

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Conformational Spectroscopic Studies of *trans*-1,2-Bromoiodocyclohexane

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We have recently reported new infrared and Raman spectral data for various monohalo[1] and *trans*-1,2-dihalocyclohexanes.[2-4] By these methods we have studied the conformational equilibrium in the liquid, in various solutions and in the crystalline states. A detailed study of the infrared and Raman spectra of these molecules has revealed a remarkable similarity between their spectra.

Whereas the *e*-conformer is the more stable in all the halocyclohexanes,[1] the *aa* conformer becomes increasingly stabilized relative to *ee* with heavier halogens in the *trans*-1,2-dihalocyclohexanes.[2-4] Thus, the dichloro derivative[2] crystallizes in *ee*, and the dibromo[2] and chloroiodo derivatives[4] crystallize in *aa*, whereas the intermediate molecule bromochlorocyclohexane is present as *ee* in the low temperature and as *aa* in the high pressure solid.[2,3]

These results have encouraged us to synthesize *trans*-1,2-bromoiodocyclohexane (BIC). However, it turned out that this molecule was quite unstable at room temperature and rapidly turned red because of free halogen. Therefore our spectral data, reported in the present communication, are not as complete as those obtained for the previous molecules.

Experimental. Hypoiodous acid (prepared from HgO and I_2) was added to cyclohexene dissolved in diethyl ether, containing 4 % water. To the formed 2-iodo cyclohexanol was added phosphoric pentabromide. After filtration, the solution was washed with KI and $NaHSO_3$ solutions, carefully dried with Na_2SO_4 and the ether evaporated. The product was distilled three times at *ca.* 60° at 1 torr. Mass spectrometric analysis gave the expected fragmentation for BIC, but revealed the existence of small impurities, notably of *trans*-1,2-dibromocyclohexane. Attempts to purify the sample further by preparative gas chromatography failed since the compound decomposed into several components upon heating.

The infrared and Raman spectrometers, the low temperature and the high pressure diamond infrared cells have been described.[3] Infrared spectra of BIC were recorded as a capillary between CsI windows, as solutions in CS_2 and CH_3CN filled into sealed cells and as a pure liquid in the low temperature cell. The sample crystallized only after prolonged cooling and annealing, undoubtedly affected by the present impurities. Crystallization under high pressure was not achieved at room temperature, but by simultaneous pressing and cooling a crystalline sample was obtained which remained crystalline upon heating to ambient temperature.

The red colour of free halogen was greatly enhanced during exposure to the laser light and effectively prevented the Raman recording. A small quantity of mercury added to the Raman cell reduced the colour and a reasonably good Raman spectrum was obtained. Dissolved in CH_3CN BIC gave a quite good Raman spectrum since the yellow colour of free iodine in this solvent did not effectively absorb the 6328 Å radiation.

Results and discussion. The infrared and Raman frequencies of BIC observed below 1500 cm^{-1} are listed in Table 1. Some of the weaker bands are undoubtedly caused by impurities, *e.g.* those at 1177, 999, 663, and 538 cm^{-1} belonging to *trans*-1,2-dibromocyclohexane.[2] Although the spectral data are somewhat incomplete, various conclusions can be drawn on comparison with the other dihalocyclohexanes.[2-4]

As expected, BIC crystallizes in the *aa*-conformation at low temperatures, since the bands, vanishing in the crystalline state, are enhanced in the polar solvent CH_3CN compared with the unpolar CS_2. The high pressure crystal, initially formed at low temperature, also remained in the *aa* conformation upon heating to room temperature.

It appears from Table 1 that the infrared and Raman bands assigned to *ee* have generally much lower intensities in the liquid state than those assigned to *aa*. Moreover, upon cooling, but before crystallization, the *ee* bands decreased further in intensities compared to the *aa* bands. Thus, there is a considerable energy difference between the conformers in the liquid state. Only a small fraction of the molecules is present in the *ee* conformation at room temperature as a result of the steric repulsion between the bulky equatorial halogens.

Only 5 definite instances were detected of infrared bands, vanishing in the crystal. This result for BIC is in striking contrast to the other *trans*-1,2-dihalocyclohexanes for which 15 – 21 vanishing bands were observed.[2-4] For dichloro-, dibromo-, bromochloro-, and chloroiodocyclohexane *ca.* 2/3 of the bands below 1400 cm^{-1} are characteristic for the *ee* or *aa* conformer while *ca.* 1/3 are coinciding bands for both conformers. In BIC, on the other hand, the vast majority of vibrational bands might be common for both conformers. However, because of the very low abundance of the *ee*-conformer in the liquid, weak *ee* bands are not detected, resulting in the small number of disappearing bands upon crystallization.

The spectral data around 1000 cm^{-1} agree perfectly with the other dihalocyclohexanes and confirm the diagnostic value of this region.[2,5] Thus, two pairs of bands were observed: 1031 and 1045 cm^{-1} (*aa* and *ee*) as well as 994 and 978 cm^{-1} (*aa* and *ee*). The latter pair of bands is particularly prominent in the infrared spectra of mono-, *trans*-1,2-, and *trans*-1,4-dihalo cyclohexanes.[2] Invariably, the *aa* (*a*) band has a higher wave number than the *ee* (*e*) band. They seem to have approximately equal extinction coefficients for both conformers [5] and are assigned as ring stretching modes.

Spectral correlations [6] in the 700 – 500 cm^{-1} region are less clear and the "C-halogen stretching bands" cannot be picked out with certainty. Tentatively, we assign the bands at 628 and 512 cm^{-1} to the *aa* C – Br and C – I bands, the former being very intense in Raman, the latter in infrared.[5] The 690 cm^{-1} band vanishing in the crystal is probably the *ee* C – Br

Table 1. Infrared and Raman spectral data of *trans*-1,2-bromoiodo cyclohexane.

Infrared		Raman	Conformer	Infrared		Raman	Conformer
Liquid	Solid −170°C	Liquid		Liquid	Solid −170°C	Liquid	
1458 m	1456 m [a]			994 vs	993 s	998 vw	*aa*
1444 s	1447 m	1442 w,bd		978 m	*	974 vw	*ee*
	1440 m			899 s	898 m	902 vw	
1431 s	1429 m			858 s	861 s	867 m	
1356 m	1355 m			838 w	*	841 vw	*ee*
1340 m	1338 m				820 w,sh		
1330 w,sh	1336 m	1333 vw	imp.	810 m	809 s	812 w	
1296 vw	1295 vw	1297 vw	*aa ee*	801 w,sh	800 w	803 w	
1279 vw				690 m	*	692 w	*ee*
1271 vw,sh				670 w			
1264 w	1266 m	1267 vw		663 m,sh	662 m		
1253 w	1253 w	1257 m					
1192 s	1186 m	1196 m		656 m	655 m	655 w	
1177 w	1171 w		imp.	651 m,sh			
1161 vs	1158 m	1165 m		628 w	629 m	632 s	
1136 m	1134 w	1134 vw	*aa ee*	615 w,sh	621 w		
1121 vw	1115 m	1117 vw		538 w	533 m		imp.
1111 w	1106 w			512 vs	505 vs	514 vw	
1071 w	* [b]		*ee*	500 m,sh	500 m,sh		
1058 w	1056 w			467 w	466 w	469 s	
1045 vw	*	1047 vw	*ee*	355 vw		357 m	
999 w,sh	998 w		imp.	309 m	308 m	308 vw	
				286 vw		286 m	
				275 vw			
						224 m	
						197 w	
						174 w	
						157 m	
						136 w	
						100 vw	

[a] Abbreviations: s, strong; m, medium; w, weak; v, very; sh, shoulder; bd, broad; imp., impurity.

[b] Bands marked with an asterisk are absent in the crystalline state.

stretching band whereas the C−I band may be very weak and coincide with one of the *aa* bands below 670 cm^{-1}.

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