Applications of Vibrational Infrared Circular Dichroism to Biological Problems: Stereochemistry of Proton Exchange in Acetoin (3-Hydroxybutan-2-one) Catalysed by Acetolactate Decarboxylase

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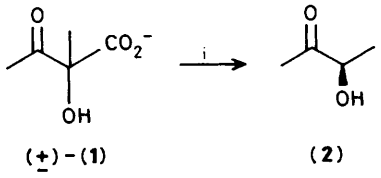
The first example is reported of the use of vibrational infrared circular dichroism to define the absolute configuration of a compound not previously described: the stereospecifically deuteriated acetoin produced on incubation of racemic acetoin with acetolactate decarboxylase.

Acetolactate decarboxylase [(*S*)-2-hydroxy-2-methyl-3-oxobutanoate carboxylyase, EC 4.1.1.5] catalyses the decarboxylation of both (*S*)- and (*R*)-α-acetolactate (2-hydroxy-2-methyl-3-oxobutanoate) (**1**) to give (*R*)-(−)-acetoin (**2**).[1,2] In the final stage of the reaction, a proton is delivered to the carbon atom to which the carboxylate group was originally attached.[3] We here report on an investigation of the reversibility and, by vibrational infrared circular dichroism, of the stereochemistry of the protonation step.

When racemic acetoin (**2**) was incubated with acetolactate decarboxylase† in D_2O solution, and the reaction followed by ^1H n.m.r. spectroscopy, the methine proton was observed to be replaced by deuterium in a process that stopped after 50% exchange. This observation strongly suggested that exchange was attributable to the reversible operation of the last step in the decarboxylation. Presumably this consists of protonation of an enediol, or an equivalent system derived from a Schiff's base, as in the acetoacetate decarboxylase reaction.[4] The observation that deuterium incorporation reached a limiting value of 50% further suggested that exchange was stereospecific and limited to the (*R*)-(−)isomer, the normal product of the decarboxylation step.[1,3]

I.r. and electronic, ordinary and c.d. spectra were obtained in carbon tetrachloride for both (*R*)-(−)-acetoin and the partially deuteriated acetoin. The electronic c.d. spectra are illustrated in Figure 1. (*R*)-(−)-Acetoin possesses a strong electronic c.d.‡ typical of a chiral ketone. The product from the enzyme reaction has a molar c.d. about 23 times weaker indicating that it is at least 'pseudo-racemic' in nature.

The vibrational c.d. (vib.c.d.) spectra§ are more revealing. In Figure 2 are shown the data in the C–D stretch region (2250—2050 cm^{-1}). Whilst the (*R*)-(−)-acetoin of normal isotopic composition does not display significant absorption



(±)-(1) (2)

i: acetolactate decarboxylase.

† The enzyme, from a *Bacillus* species, was kindly provided by Novo Industri A/S.

‡ Determined on a JASCO J40 CS spectropolarimeter.

§ Determined on a spectrometer constructed by A.F.D. (A. F. Drake, *J. Phys. E. Sci. Instrum.*, 1986, **19**, 170), for solutions in CCl_4. Baselines were obtained using racemic acetoin.

the partly deuteriated acetoin has three absorption bands each with an associated negative c.d. The proton–deuterium exchange had clearly been stereoselective. The observation of three absorptions associated with a single deuteriation is at first surprising. However, similar results have been reported[5] and they derive presumably from overtones and Fermi resonance. The acetoin product of the exchange reaction is, therefore, not racemic.

The 'pseudo-racemic' composition of the enzymatically derived product was confirmed by the absence of vib.c.d. associated with its O–H stretching vibration in contrast to (*R*)-(−)-acetoin (Figure 3).

In Figure 4 are presented the ordinary i.r. and vib.c.d. spectra in the C–H stretch region. The 'pseudo-racemic' product displays positive vib.c.d. under the C–H stretch absorption whereas the corresponding dichroism for (*R*)-(−)-

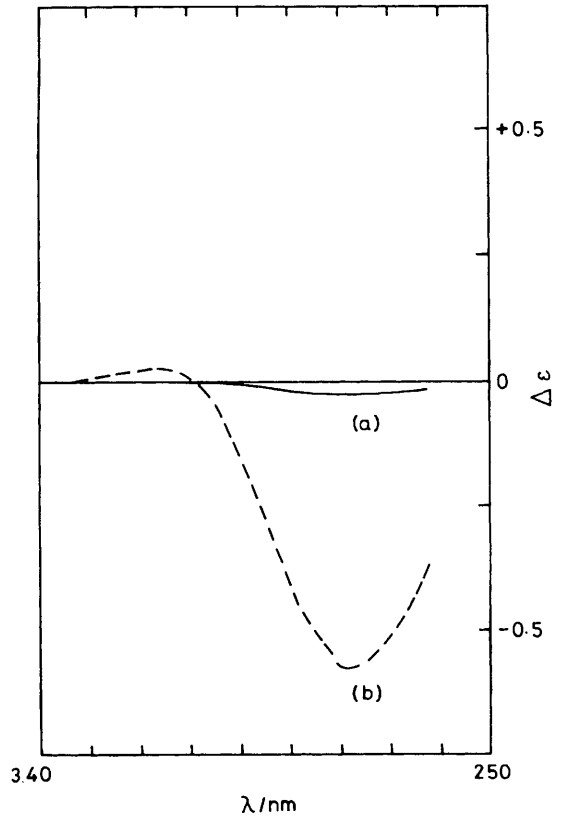


Figure 1. The electronic c.d. in CCl_4 of (a) 'pseudoracemic' acetoin; (b) (*R*)-(−)-acetoin (**2**).

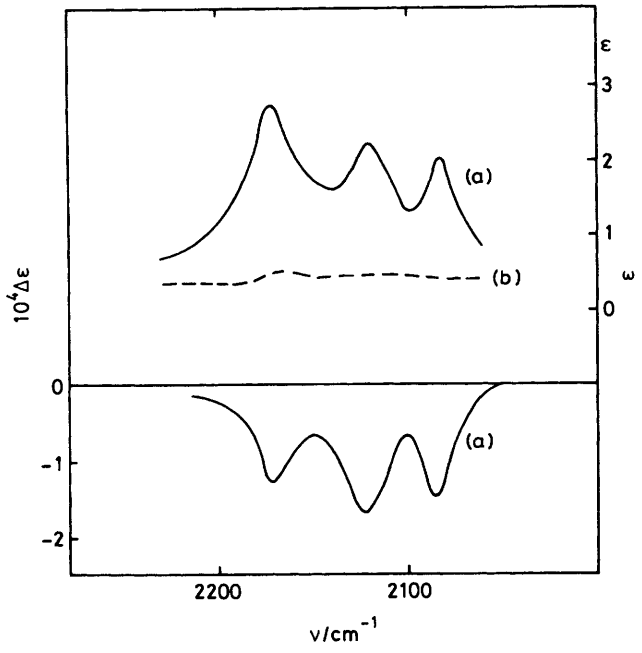


Figure 2. The C–D stretch vibrational absorption and c.d. in CCl_4 of (a) 'pseudoracemic' partly deuteriated acetoin; (b) (*R*)-(−)-acetoin (**2**).

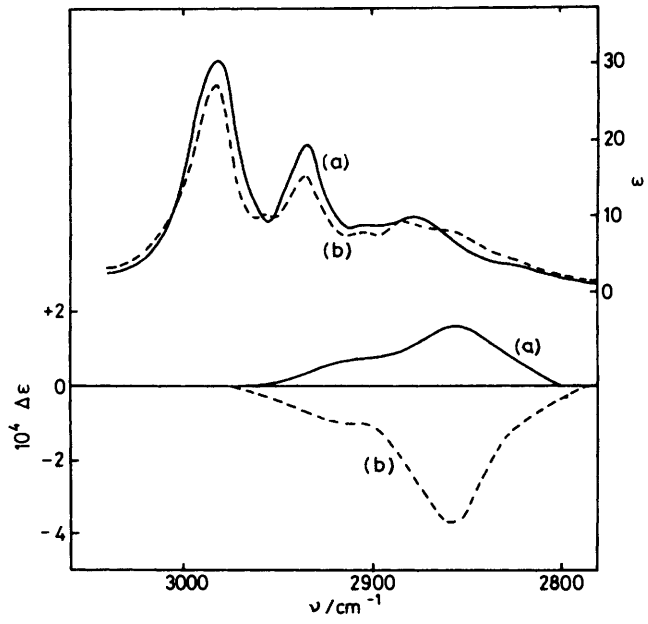


Figure 4. The C–H stretch vibrational absorption and c.d. in CCl_4 of (a) 'pseudoracemic' partly deuteriated acetoin; (b) (*R*)-(−)-acetoin (**2**).

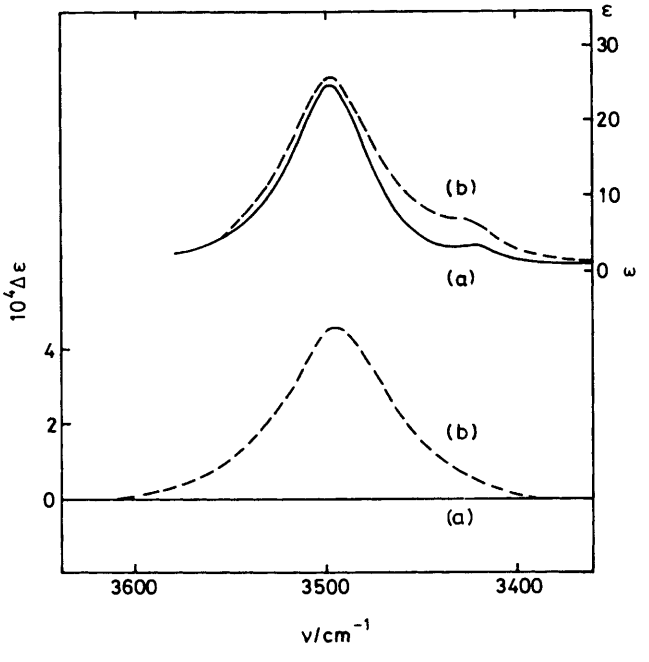


Figure 3. The O–H stretch vibrational absorption and c.d. in CCl_4 of (a) 'pseudoracemic' partly deuteriated acetoin; (b) (*R*)-(−)-acetoin (**2**).

acetoin of normal isotopic composition is negative in sign. This proves unambiguously that the non-deuteriated component of the 'pseudo-racemic' product has the (*S*)-configuration and consequently that the deuteriated component has the same configuration (*R*) as the (−)-acetoin. The intensity of the C–H vib.c.d. of the 'pseudo-racemic' product is approximately half that of the normal (*R*)-(−)-isomer, consistent with the inference, based on ^{1}H n.m.r. observations made during the enzymatic experiment, that only one enantiomer of the racemic acetoin had undergone exchange. These conclusions are also necessary to explain the paucity of optical activity in the electronic c.d. and the O–H stretch vib.c.d. for the enzymatically derived product and the need to adopt the term 'pseudo-racemic.'

By studying the variation with dilution of the vib.c.d. spectra it was shown that these are not influenced by intermolecular effects.

It can therefore be concluded that acetolactate decarboxylase in D_2O induces a D/H exchange in (*R*)-acetoin and not the (*S*)-isomer to give a deuteriated product with retention of configuration. These results demonstrate the applicability of vibrational i.r. c.d. to the study of the stereochemistry of biological processes. They also show that both qualititative and quantitative data can be obtained using this technique.

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