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Note

Fourier transform infrared detection in reversed-phase high-performance liquid chromatography of metallocene–amino acid adducts

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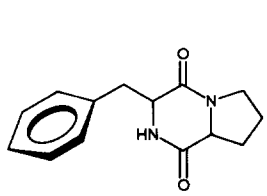
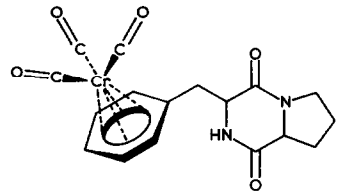
We have recently shown that a $Cr(CO)_3$ ligand could be incorporated on the π-electrons of the side-chain of aromatic amino acids by refluxing with $Cr(CO)_6$ in water-containing media[1]. This observation is in complete opposition to previous reports that emphasized the necessity for using dehydrated solvents during this kind of reaction[2].

Some of the adducts that are formed are fairly stable and could be crystallized, *e.g.*, the diketopiperazine derivative II.



I



II

In other cases, although we observed in the reaction medium the appearance of a yellow colour, which usually indicates the formation of an adduct, we were not able to isolate it. However, the presence of the adduct may be confirmed by a strong UV absorption during the reversed-phase high-performance liquid chromatography (HPLC) of the reaction medium.

As the formation of a $Cr(CO)_3$ adduct is known to induce two strong absorptions in the IR spectrum[3] corresponding to the CO stretching modes, as confirmed with various crystalline adducts (Fig. 1), IR detection was used during the HPLC procedure in order to obtain more information on the chemical structures of the eluted material.

EXPERIMENTAL

The high-performance liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) consisted of a Model 6000 A pump and a Model U6K injector with a Waters Assoc. μBondapak C_{18} column (30 × 0.39 cm I.D.; particle size = 10 μm). The eluates were successively detected with a Model 450 variable-wavelength detector set

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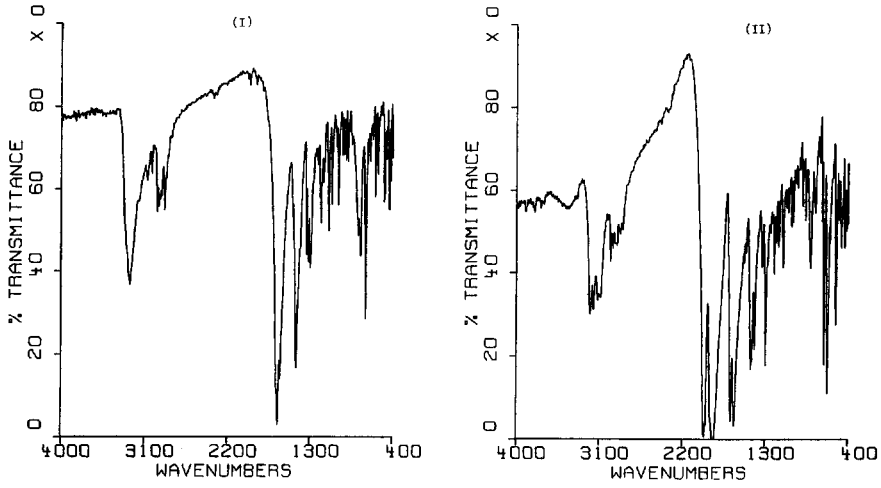


Fig. 1. IR spectra of the diketopiperazine derivative I and its $Cr(CO)_3$ adduct (II).

at 254 nm and a Nicolet 7199 B Fourier transform infrared (FTIR) spectrometer fitted with a Zn–Se Cell (path length 0.05 mm). IR detection during reversed-phase HPLC is limited by the strong absorptions that occur with the commonly used eluents. Examination of the IR spectrum of the two further defined elution systems showed that IR detection is limited to two main spectral windows: 1750–2050 and

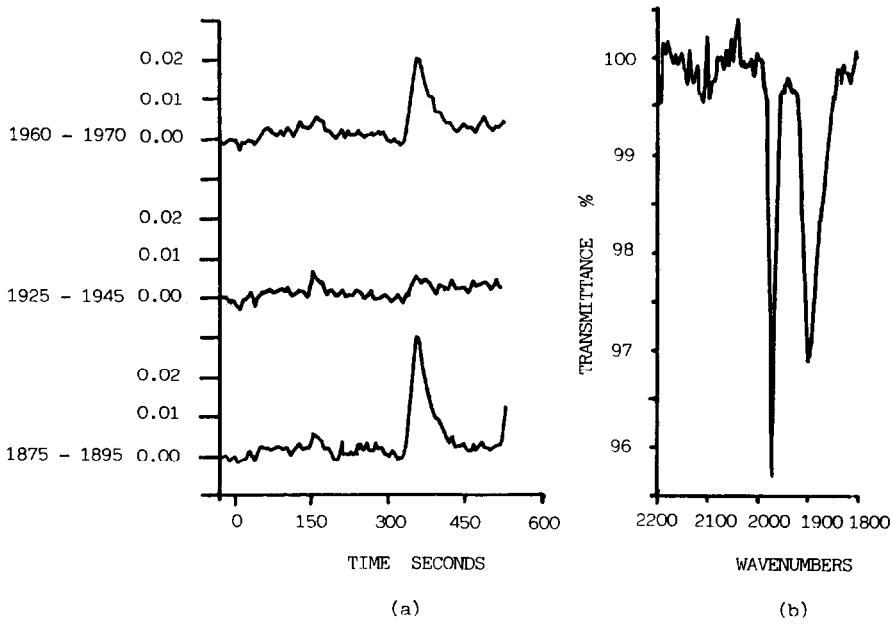


Fig. 2. FTIR detection of the $Cr(CO)_3$ adduct of CH_3CO–Phe–OCH_3 during reversed-phase HPLC. (a) FTIR-detected chromatogram; (b) characteristic IR absorbances.

2350–2800 cm^{-1}, thus allowing the detection of the two carbonyl-characteristic absorptions.

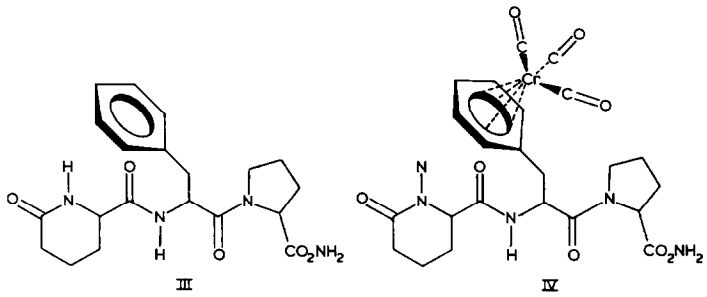
The transmittance was simultaneously measured at three wavelengths. Two of them (1895–1900 and 1965–1970 cm^{-1}) were chosen because they corresponded to the ν_{CO} bands. When an absorption was detected at these wavelengths, the complete IR spectrum of the eluate was recorded and calculated. The third wavelength (1940–1950 cm^{-1}) was used as a reference.

RESULTS AND DISCUSSION

In order to check the validity of the method, we first attempted IR detection after the reaction of CH_3CO–Phe–OCH_3 with $Cr(CO)_6$ [tetrahydrofuran–water (1:5); 100°C, 10 h]. In this instance, the corresponding adduct had been previously isolated and characterized[1]. A 100-μl volume of reaction medium was injected (corresponding to *ca.* 0.7 mg of starting material). The column was eluted at 1.5 ml min^{-1} with methanol–water–0.05 *M* phosphate buffer (pH 2.5) (45:50:5).

The results are shown in Fig. 2: two peaks appeared at the carbonyl wavelengths, whereas no absorption was observed at the reference wavelength. The complete spectrum of the IR-absorbing material (Fig. 2b) was then calculated and characteristic carbonyl absorptions displayed. The same result was obtained when a solution of the pure adduct was injected.

In the case of PCA–Phe–Pro–NH_2(III), an analogue of thyrotropin-releasing hormone (TRH) having a phenylalanine in the 2-position, we had not been able to isolate the $Cr(CO)_3$ adduct (IV). After refluxing III with $Cr(CO)_6$ in water–tetrahydrofuran (4:1) for 20 h, 100 μl of reaction medium (corresponding to *ca.* 0.7 mg) were injected. The column was eluted at 1.5 ml min^{-1} with acetonitrile–water–0.05 *M* phosphate buffer (pH 2.5) (27:68:5).



III IV

The IR absorptions of the eluate are shown in Fig. 3. At the same time at which a strong UV absorption occurred, we observed two peaks at the carbonyl wavelengths whereas no absorption was detected at the reference wavelength. The spectrum of this product could be calculated (Fig. 3b) and displayed characteristic carbonyl absorptions. It could thus be concluded that, although in this instance we had not been able to isolate it, the $Cr(CO)_3$ adduct of III was formed and localized during elution.

In conclusion, we have demonstrated here the possibility of using IR detection during reversed-phase HPLC. This allows structural informations on sensitive eluting

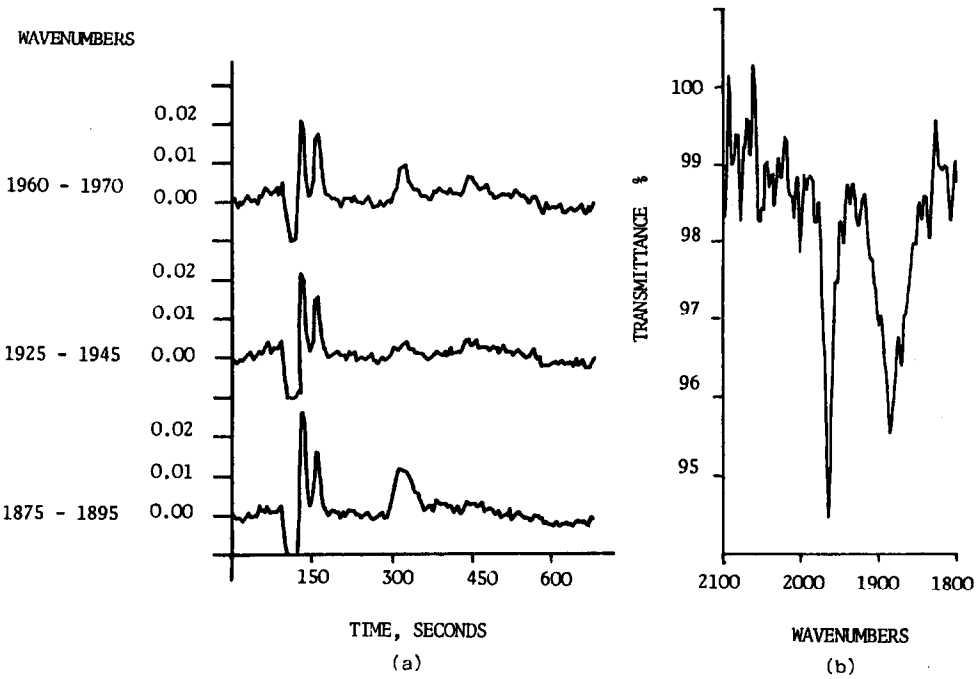


Fig. 3. FTIR detection of the $Cr(CO)_3$ adduct (IV) of PCA-Phe-Pro-NH_2(III) during reversed-phase HPLC. (a) FTIR-detected chromatogram; (b) characteristic IR absorbances.

materials to be obtained without having to isolate them, but it must be emphasized that the position and the narrowess of the spectral windows available limit the number of possible applications considerably.

REFERENCES

1 C. Sergheraert, J. C. Brunet and A. Tartar, *J. Chem. Soc., Chem. Commun.*, (1982) 1418.
2 C. A. L. Mahaffi and P. L. Pauson, *Inorg. Synth.*, 19 (1979) 154.
3 L. J. Bellamy, *Infra-Red Spectra of Complex Molecules*, Wiley, New York, 1966, p. 130.