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Short communication

Application of polarimetric detector for the high-performance liquid chromatographic determination of the optical purity of 5(4*H*)-oxazolones

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Abstract

An HPLC procedure for monitoring one of the reactive intermediates in peptide synthesis, 5(4*H*)-oxazolone, was developed. The reversed-phase mode made it possible not only to monitor the formation and consumption of oxazolone, but also to determine the concentration of other components of the reaction mixture. Moreover, with polarimewtric and UV detectors coupled in series it was possible to determine the content of both oxazolone enantiomers in the reaction mixture. Hence with the achiral chromatographic system employed, the enantiomeric excess of the crucial component of the mixture could be monitored.

1. Introduction

The main objective of peptide synthesis is the preparation of peptides not only chemically pure but also of defined chiral identity. The well known thalidomide tragedy [1] still remains a classic warning of how dangerous a drug contaminated with its diastereomeric counterpart can be. Taking into consideration the large production scale of some peptides, any improvement in the reaction yield and the purity of the product is of great economic importance. A great deal of effort has been devoted to trying to define the circumstances under which racemization can be eliminated or minimized. In general, any activation of an N-protected amino acid or a peptide indispensible for peptide bond formation is accompanied by a risk of the loss of chiral purity of the activated moiety. As the formation of 2-alkyl-5(4*H*)-oxazolones intermediate is commonly regarded as one of the main roots of the racemization process, an analytical technique for monitoring their transformation in peptide synthesis is very desirable. 5(4*H*)-Oxazolones easily undergo base-catalysed racemization and their subsequent aminolysis by the amino component contributes to peptide racemization. Our previous findings [2], along with many other results [3,4], indicate that aminolysis of racemized 5(4*H*)-oxazolones is responsible for slow peptide racemization with a prolonged reaction time, but the question arises of whether they are formed already racemized or whether they are initially chirally pure and racemize during the reaction. This paper describes the application of

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an HPLC method for monitoring the formation of oxazolone and its transformation.

2. Experimental

2.1. Apparatus and reagents

An HP 1090A chromatograph equipped with a diode-array UV detector and a system consisting of a Pye Unicam PU 410 pump, PU 4020 variable-wavelength UV detector, Knauer Chiralyser 2 polarimetric detector and Kipp Zonen BD 41 recorder were employed. Separations of 5(4*H*)-oxazolones and other components of the investigated reaction mixtures were performed on a Nucleosil ODS 100-5 column (Macherey–Nagel) with aqueous acetonitrile or methanol as the mobile phase. Tetrahydrofuran (THF) was distilled from above Na–K alloy. Water was distilled twice in glass apparatus. Methanol and acetonitrile (Baker, chromatographic grade) were used as received. For detailed chromatographic conditions, see Figs. 1 and 2.

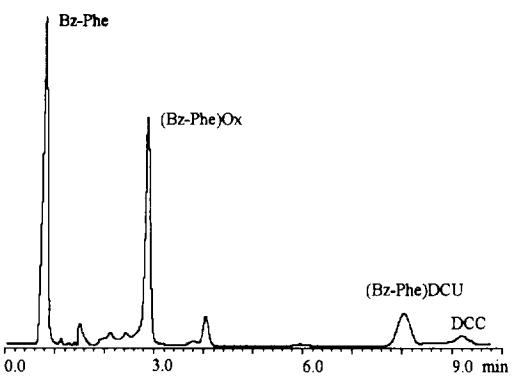

Fig. 1. Chromatogram of the reaction mixture of Bz-Phe with DCC. Reagent concentrations: Bz-Phe, 5.2 m*M*; DCC, 5.1 m*M*. Column, Nucleosil ODS 100-5 (100 × 4.6 mm I.D.); mobile phase, methanol–water (75:25); flow-rate 1.0 ml/min; temperature, 35°C; detection, UV at 220 nm. Reaction:

$$\text{Bz-Phe} + \text{DCC} \xrightarrow{\text{THF, 20°C}} \text{(Bz-Phe)Ox} + \text{(Bz-Phe)DCU}$$

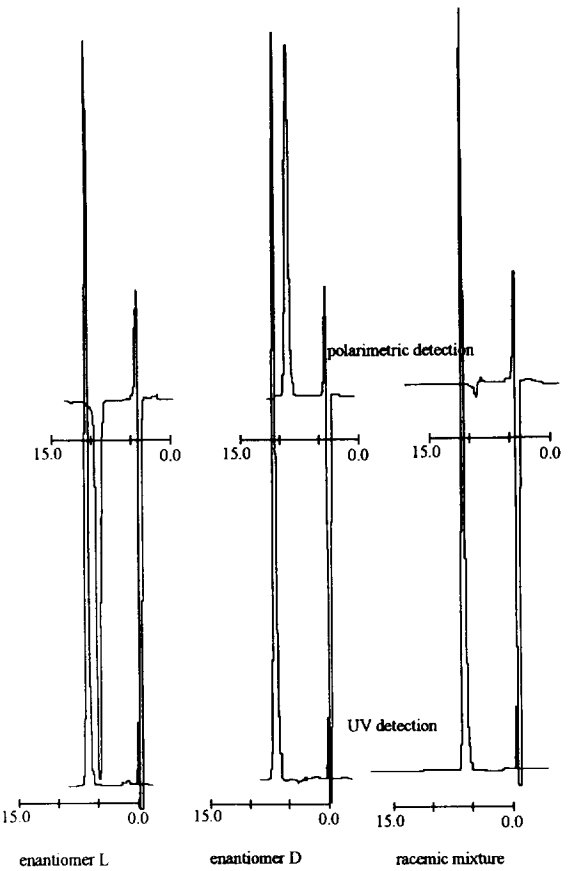

Fig. 2. Chromatograms of the enantiomers of 2-phenyl-4-benzyl-5(4*H*)-oxazolone. Column, Nucleosil ODS 100-5 (100 × 4.6 mm I.D.); mobile phase, acetonitrile–water (60:40); flow-rate 1.0 ml/min; detection, UV at 285 nm and polarimetric.

2-Phenyl-4-benzyl-5(4*H*)-oxazolone (Bz-Phe)-Ox was obtained from N-benzoylphenylalanine and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline according to the published procedure [5]. Both the D and L-enantiomers were prepared.

Owing to the possibility of oxazolone autoracemization [6], the oxazolone standards for detector calibration were prepared by dissolving the pure enantiomers in acetonitrile just before use.

N - Benzoylphenylalanyl - N,N′ - dicyclohexylurea (Bz-Phe)DCU was obtained from N-benzoylphenylalanine (Bz-Phe) and N,N′-dicyclo-

hexylcarbodiimide (DCC) according to the published procedure [7]. Both the D- and L-enantiomers were prepared.

2.2. Reaction conditions

The reactions were carried out in thermostated vessels with magnetic stirring. All the reagents were added as solutions in an appropriate solvent (for the initial concentrations and the reaction temperature, see Fig. 4). The resulting reaction mixtures were injected directly in the HPLC system.

3. Results and discussion

Previously, we reported the use of Pirkle and Oi chiral stationary phases for the normal-phase separation of protected dipeptides and N-acyl-N,N′-dicyclohexylurea enantiomers in carbodiimide-mediated peptide synthesis [7,8]. An attempt to apply those stationary phases to the separation of 2-alkyl-5(4*H*)-oxazolone enantiomers failed and prompted us to search for a different method for their rapid analysis.

Although 2-alkyl-5(4*H*)-oxazolones are susceptible to hydrolysis, they are stable under the conditions of reversed-phase chromatography. Good resolution of all chemical species present in the reaction mixture was achieved using the conditions applied (Fig. 1) and, together with the use of polarimetric and UV detectors coupled in series, enabled us to measure the enantiomer ratio in the 5(4*H*)-oxazolone mixture. To make this possible, the UV and polarimetric signals had to be evaluated simultaneously [9] as the UV detector provided information on the total oxazolone concentration whereas the polarimetric detector gave the signal proportional to the enantiomeric excess (Fig. 2).

The reliability of such a chromatographic system was successfully tested by comparison of the data on the chiral purity of 5(4*H*)-oxazolone standards obtained using HPLC and conventional polarimetric methods. The straight regression line of the experimental data ($R^2 = 0.995$) proved that the oxazolone remains chemically and chirally intact under the chromatographic conditions applied (Fig. 3). The detection limit for each of the 2-phenyl-4-benzyl-5(4*H*)-oxazolone enantiomers was 2 μg on-column and the linear response range was 0.5–20 m*M*.

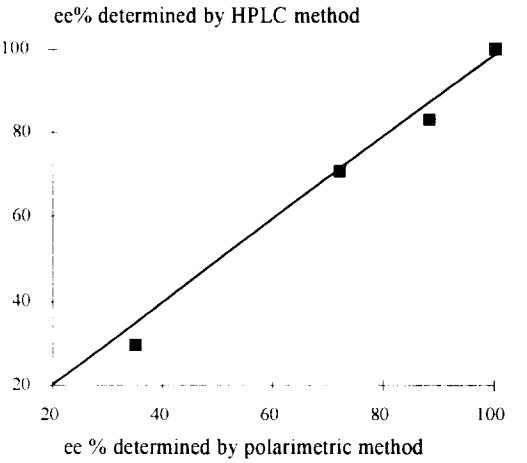

Fig. 3. Comparison of the data on chiral purity of 5(4*H*)-oxazolone standards obtained using HPLC and conventional polarimetric methods. ee = Enantiomeric excess.

The method was applied to the investigation of the reaction of dicyclohexyl carbodiimide with benzoylphenylalanine as a model reagent. The compound was chosen for its racemization-prone properties and the fact that crystalline 2-phenyl-4-benzyl-5(4*H*)-oxazolone derived from benzoylphenylalanine is chemically and chirally stable and its physico-chemical and optical data are well known. As the oxazolone concentration was very low at the beginning of the reaction, the first determination of its chiral purity could be performed not earlier than a few minutes after the start of the reaction. A specific interdependence was observed: the longer the reaction time, the lower was the chiral purity of the oxazolone. Extrapolation of the experimental data gave a 100% optical purity of the initially formed oxazolone (Fig. 4). Although this value has no direct physical meaning, it allows for the deduction that 2-alkyl-5(4*H*)-oxazolones are formed during peptide synthesis without altering the chirality of the α-C atom of the activated

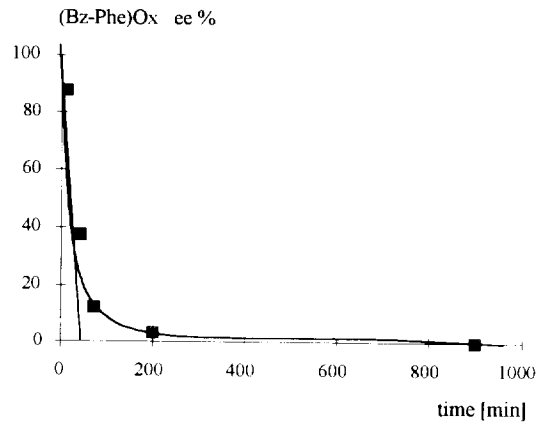

Fig. 4. Change in chiral purity of 2-phenyl-4-benzyl-5(4*H*)-oxazolone during the course of the reaction of DCC with Bz-Phe. Reagent concentrations: Bz-Phe, 15.1 m*M*, DCC, 14.8 m*M*. Reaction:

$$\text{Bz-Phe} + \text{DCC} \xrightarrow{\text{THF, 20°C}} \text{(Bz-Phe)Ox} + \text{(Bz-Phe)DCU}$$

N-acylamino acids and they racemize slowly in the course of the reaction.

Acknowledgements

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