A STUDY ON THE STEREOCHEMICAL PURITY OF TRANDOLAPRIL AND OCTAHYDRO–1*H*–INDOLE–2–CARBOXYLIC ACID BY HPLC METHOD

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Abstract: HPLC conditions for the identification of stereoisomers and stereochemical purity of the key intermediate in Trandolapril synthesis, octahydro–1*H*–indole–2–carboxylic acid, and final drug were elaborated. The chemical and stereochemical purity of synthetic Trandolapril was proved to be as high as 99.3–99.8%, on both non chiral and chiral RP – columns.

Keywords: octahydro–1*H*–indole–2–carboxylic acid, trandolapril, HPLC

Trandolapril, (3a*R*,7a*S*)–1–[N–1(*S*)–(ethoxycarbonyl)–3–phenylpropyl]–(*S*)–alanyl]octahydro–1*H*–indole–2(*S*)–carboxylic acid (see, Figure 3), is an angiotensin converting enzyme (ACE) inhibitor widely used as an efficient orally active antihypertensive drug. It is specially recommended for the treatment of arterial hypertension in patients after myocardial infarction with dysfunction of the left heart ventricle (1). The synthesis of this generic drug has been patented in 1981 by Hoechst (2) and no more synthetic details was later described.

The key intermediate in trandolapril synthesis is (2*S*,3a*R*,7a*S*) – octahydro–1*H*–indole–2–carboxylic acid (II). This compound, however in racemic form, is prepared by catalytic hydrogenation of the corresponding imine–acid salt (I) (Figure 1).

According to the patent literature (2) the main product of this hydrogenation, carried out on Pt ((2), general description) or on a Pd catalyst ((2), experimental), is a racemic stereoisomer (IIA). We found that this reaction, independently which catalyst was used, gave a mixture of two major diastereoisomers (IIA) and (IIB). By changing conditions of the reduction (type of catalyst, solvent, temperature, acid addition etc.) and by further recrystallizations we were able to prepare both diastereoisomers in an almost pure form. The determination of the stereochemical structures of both products was of importance because only the correct stereoisomer could be used for the synthesis of the drug.

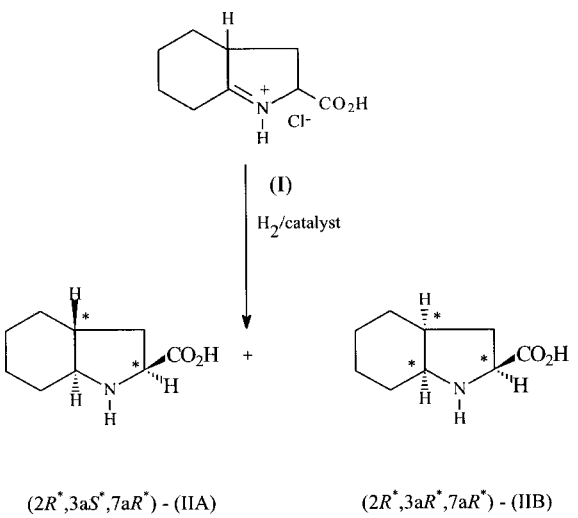

Figure 1. Scheme of octahydro–1*H*–indole–2–carboxylic acid synthesis.

HPLC technique was succesfully used for the identification of the diastereoisomers. We elaborated RP–HPLC conditions (see Experimental) in which we could observe both diastereoisomers. The first diastereoisomer eluted with the retention time (t_R) 3.9 min, identical to the retention time of commercialy available 2*S*,3a*S*,7a*S*–acid (II) (sold as L–octahydro–1H–indole–2–carboxylic acid; Bachem) indicated that it was racemic (2R^*,3aR^*,7aR^*)–acid (IIB), so the second peak with the retention time 4.2–4.4 min corresponded to racemic (2R^*,3aS^*,7aR^*)–acid (IIA), which was further proved by the total synthesis of trandolapril. HPLC allowed to check the purity of our synthetic octahydro–1*H*–indole–2–carboxylic acid, and to follow the process of enhancement of synthetic product into the correct racemic diastereoisomer (Figure 2.).

The content of diastereoisomers was determined directly on a C–18 column as well as indirectly *via* preparation of (*N*–quinol–6–yl)–carbamoil–derivatives of octahydro–1*H*–indole–2–carboxylic acids and their HPLC resolution (AccQ·Tag Amino Acid Analysis Method (3)). This methodology was developed and recommended for the determination of amino acid mixtures by Milipore Corporation Waters (4), but was never used for octahydro–1*H*–indole–2–carboxylic acids. We found that derivatization of a diastereoisomeric mixture of (II) run smoothly and the recommended method gave good separation of diastereoisomers and was convenient for the determination of diastereomeric ratio.

The results obtained by both methods are roughly consistent (Table 1) but the direct method is much simpler and much more reliable. Octahydro–1*H*–indole–2–carboxylic acid has no UV absorption, so with the simple direct method a refractive index detector must be used. For indirect measurements a fluorescence detector (emission wavelength for these derivatives – 395 nm) or a typical UV–detector can be used (excitation wavelength – 250 nm).

Table 1. Contents of diastereoisomeric octahydro-*1H*-indole-2-carboxylic acid (%) in different samples by direct and indirect methods

Sample	Direct HPLC (refractive index detector)		Indirect HPLC derivatization (UV detector)	
	(2*R**,3a*S**,7a*R**)	(2*R**,3a*R**,7a*R**)	(2*R**,3a*S**,7a*R**)	(2*R**,3a*R**,7a*R**)
1	91.8	6.9	92.3	7.7
2	91.1	7.5	92.3	7.7
3	70.8	23.0	75.8	22.6
4	33.7	51.9	27.1	66.4
5	38.4	61.6	36.2	61.1

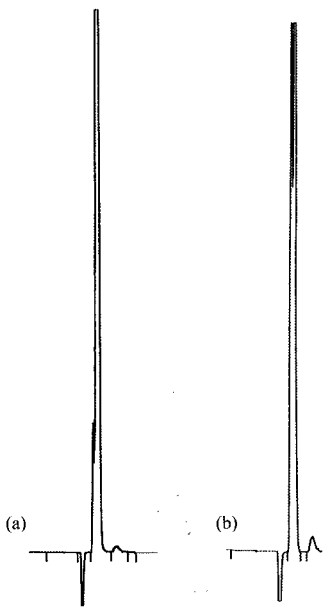

Figure 2. Typical chromatograms of octahydro-1*H*-indole-2-carboxylic acids. (a) after enrichment: 96.4% (2*R**,3a*S**,7a*R**) – (IIA), retention time (t_R = 4.3 min) + 3.6% (2*R**,3a*R**,7a*R**) – (IIB), (t_R = 3.9 min); (b) crude material: (2*R**,3a*S**,7a*R**) – (IIA) + 33.6% (2*R**,3a*R**,7a*R**) – (IIB).

In further steps of Trandolapril synthesis, (2*R**, 3a*S**,7a*R**)-acid (II) was transformed into its benzyl ester and coupled with [1(*S*)-(etoxycarbonyl)-3-phenylpropyl]-(*S*)-alanine to give a mixture of diastereoisomers which was chromatographically resolved on a silica gel column. Separate hydrogenation of benzyl esters obtained gave trandolapril [((3a*R*,7a*S*)-1-[*N*-1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl]octahydro-indole-2(*S*)-carboxylic acid] and the second diastereisomer e.g. [((3a*S*,7a*S*)-1-[N-1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl]octahydro-indole-2(*R*)-carboxylic acid] (Figure 3).

The assignement of the stereochemical structure of both products was carried out as follows. Trandolapril was isolated from the drug Gopten® from Knoll. Both diastereoisomers were recrystallized to remove eventual traces of different diastereoisomers (if not completely resolved during column chromatography of benzyl esters).

The stereochemical purity of the final product was checked carefully using HPLC technique. HPLC conditions were elaborated, both on the C-18 RP column and on the chiral column (Cyclobond™ 2000, cyclodextrin) (see Experimental), to observe both stereoisomers. In the chosen conditions, the recrystallized trandolapril samples from different synthetic batches

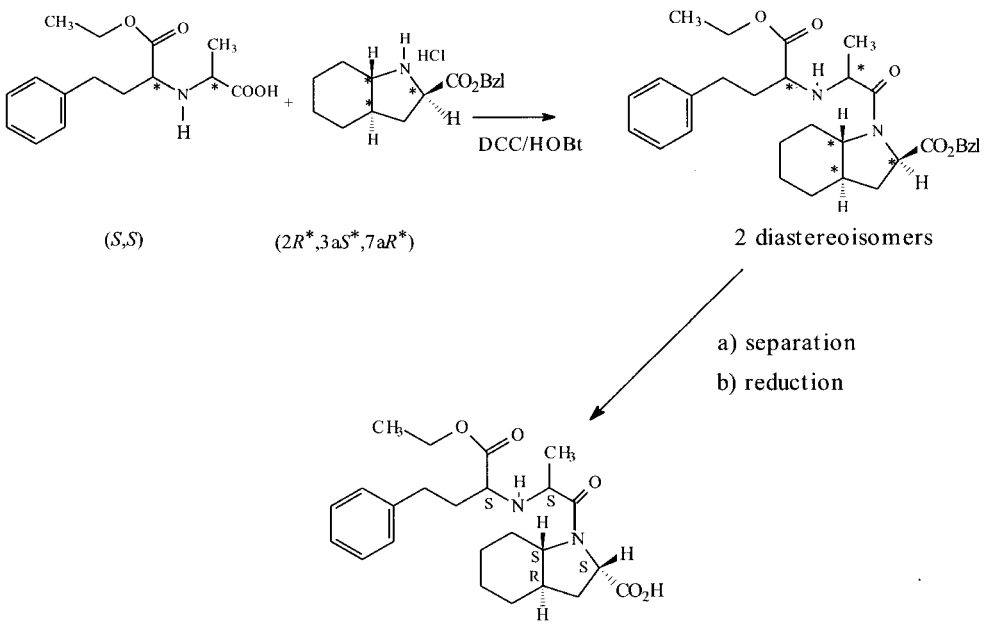

Figure 3. Scheme of last steps in trandolapril synthesis.

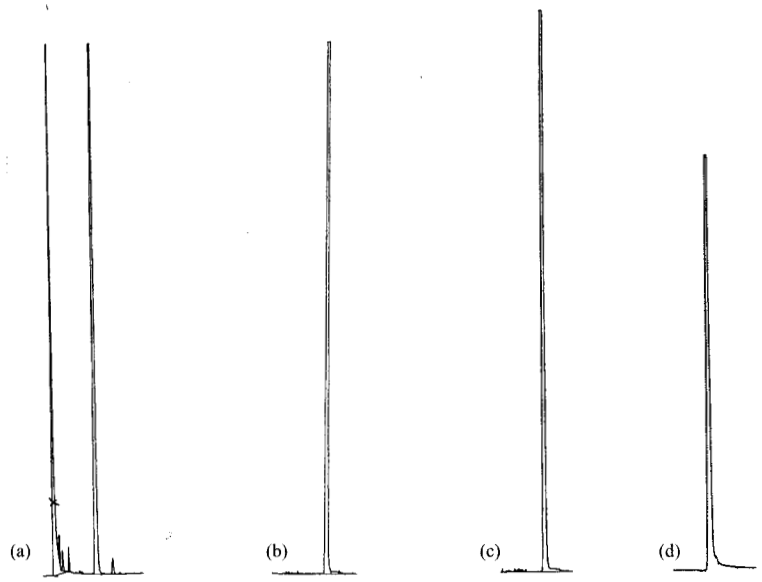

Figure 4. HPLC chromatograms of trandolapril. (a) trandolapril isolated from Gopten®, t_R = 9.1 min; co–injection with synthetic material, t_R = 9.2 min; (c) synthetic material on Waters Symmetry RP–18 column, 99.2%, t_R = 9.4 min; (d) synthetic material on Chiral Cyclobond I ™ column, 99.96%, t_R = 3.8 min.

were analysed. The retention time (t_R) was found to be 9.0–9.5 min, and the purity of Trandolapril samples was in the range of 99.3–99.8%. Co–injection with Trandolapril isolated from Gopten® gave in separable one peak at 9.2 min.

We also found that diastereoisomeric product eluted earlier from the column, and its co–injection with synthetic Trandolapril gave two well separated peaks. In this way we have shown that synthetic Trandolapril was stereochemically pure, identical with the sample isolated from the drug Gopten® (Knoll), and different from separately synthesized diastereoisomers. The stereochemical purity of Tandolapril was also confirmed by HPLC on the chiral column (Figure 4).

Experimental section describes the HPLC method which is suitable and simple for the determination of stereochemical purity of octahydro–1H–indole–2–carboxylic acid and Trandolapril.

EXPERIMENTAL

Apparatus and reagents

1. Shimadzu HPLC system consisted of: LC–10AD pomp, spectrophotometric (UV/VIS) and RID–10A refractive index detectors, degaser and Rheodyne Model Injector 20 µl.

2. Waters HPLC system consisted of: 600S Controller – pump, Tunable Absorbance Detector 486, Autosampler 717Plus, and Chromatography Manager Millenium wersion 2.15.01 software for PC computation.

3. All solvents were of HPLC–grade; all chemicals were from Fluka exept L–octahydroindole–2–carboxylic acid (from Bachem); Gopten® (capsules á 2 mg of trandolapril) from Knoll.

4. Tag® Chemistry Package containing AQC reagent for the preparation of N–[(quinol–6–yl)–carbamoil]– derivatives, C–18 AccQ Tag column 150 x 3.9 mm, 4 µm, and reagents for preparation of mobile phases were supplied by Milipore Co.

Chromatographic conditions for direct HPLC analysis of octahydro–1*H*–indole–2–carboxylic acid

Liquid chromatograph (Shimadzu) – column: Luna C18, Phenomenex, 250 x 4.6 mm, 5 µm, mobile phase MeOH + 0.005M tetrabutylammonium phosphate (45:55, v/v), flow rate 1 ml/min, injection 20 µl; refractometric detector. Retention times are presented in Figure 2.

Chromatographic analysis of octahydro–1*H*–indole–2–carboxylic acid by ACCQ·Tag Method

Derivatization of different samples of octahydro–1*H*–indole–2–carboxylic acid (standard 2*S*,3a*S*, 7a*S*–acid, crude mixtures obtained from hydrogenation, materials from enrichment procedures etc.), preparation of eluents and gradients, column separation and determination of diastereisomer contents was carried out according Milipore Instruction Manual (4) using Tag® Chemistry Package.

Chromatographic conditions for HPLC analysis of synthetic trandolapril on an RP column

Conditions (a) – column: Waters Symmetry Shield RP 18.5 µm, 4.6 × 250 mm, flow rate 1 ml/min, inj. 10 µl; detection: λ 220 nm. Phase A: 30% CH_3CN/0.087 M $NH_4H_2PO_4$. Phase B: 60% CH_3CN/ 0.087 M $NH_4H_2PO_4$, isocratic mode: 58% B; retention time (t_R) 9.0–9.5 min.

Isolation of trandolapril from Gopten®

Five capsules of Gopten® (Knoll, á 2 mg of trandolapril) were crushed and extracted overnight white stirring with aqeous methanol (0.5 mL H_2O + 15

mL MeOH). After removal of the insoluble material by filtration and evaporation of the filtrate in vacuum, the gelatinous mass was obtained. This material was analysed by HPLC in conditions (a). The HPLC method revealed (see Figure 4) the presence of some impurities and one main peak corresponding to trandolapril (retention time 9.1 min).

Chromatographic conditions for HPLC analysis of trandolapril on chiral column

Liquid chromatograph (Shimadzu) – column: Cyclobond™ 2000 (β–cyclodextrin), 250 × 4.6 mm, 5 μm, mobile phase CH_3CN + + 0.087 M $NH_4H_2PO_4$ tetrabutylammonium phosphate (40:60, v/v), flow rate 1 ml/min, injection 20 μl; λ 220 nm. Retention time for trandolapril 3.8 min, purity, 99.96%.

RESULTS AND CONCLUSION

HPLC was found to be an excellent technique to follow the process of antihypertensive drug – trandolapril production. We elaborated HPLC conditions for the identification of stereoisomers of the key intermediate in this synthesis – octahydro–1*H*–indole–2–carboxylic acid, and also to control the process of enrichment of crude synthetic octahydro–1*H*–indole–2–carboxylic acid into a correct isomer, which was taken for further synthesis of trandolapril. We also worked at the HPLC conditions for the determination of chemical and stereochemical purity of trandolapril. We could proved that the synthetic trandolapril produced at the Pharmaceutical Research Institute had a high purity (99.3–99.8%) and was identical with the trandolapril isolated from the commercial drug Gopten® and contained no diastereoisomeric impurities.

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SYNTHESIS, PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITY OF 2,6–DISUBSTITUTED 7–METHYLPURINES AND THE 7,9–DIALKYL ANALOGUES

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Abstract: The preparation of several derivatives of 2,6–disubstituted–7–methylpurines and the 7,9–dialkyl analogues possessing a potential cytotoxic or cytostatic activity has been described. These compounds were tested for biological activity using a method for the determination of the toxic effects of chemical compounds on the growth of green alga *Chlorella vulgaris*, Beijerinck 1890. The effective concentration (EC_{50}) was calculated.

Keywords: 2,6–disubstituted–7–methylpurines and the 7,9–dialkyl analogues, green alga *Chlorella vulgaris*, effective concentration EC_{50}

Some of the sulfur and halogeno derivatives of purines as mercaptopurine, thioguanine, fludarabine and azathioprine are used as anticancer and immunosupressive drugs. Some of 2–amino– or 2–alkylamino–6–methoxy–7,9–dimethylpurinium and the 7,9–dimethylguaninium salts, isolated from *Heterostemma brownii* Hay (Asclepiadaceae), exibited cytotoxic activity towards several tumor cell lines (1).

The unicellular green alga *Chlorella vulgaris* can be exploited to initial selection of chemical compounds in terms of their biological activity (cytostatic activity and cytotoxic activity). These investigations as short–term bioassay give rapid information not only about general biological activity but they can also indicate a phase of cell life cycle when the substance is the most active (2,3). The estimated dose–answer relationship for chemical compounds and calculated EC_{50} value can be correlated with physicochemical properties.

EXPERIMENTAL

Synthesis

2,6–Disubstituted–7–methylpurines **1a–c** were prepared in a simple way from 2,6–dichloro–7–methylpurine according to the described procedure (4), 2–methylthio–6–methoxy–7–methylpurine **1d** was prepared