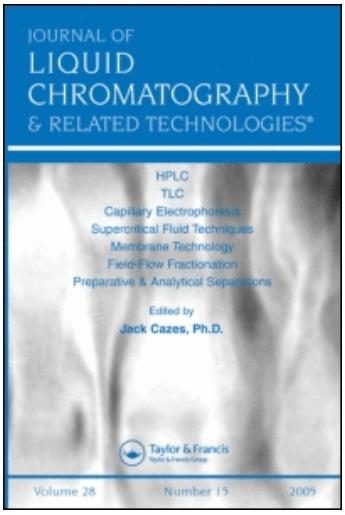

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ABNORMAL RETENTION OF SOME DIHYDROGENOPHOSPHATE-1-BENZOYL-ISOQUINOLINE IONS PAIRS EXCEPTIONALLY STABLE OBSERVED IN LIQUID CHROMATOGRAPHY

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ABSTRACT

Abnormal retention of some dihydrogenophosphate-1-benzoylisoquinoleine ions pairs is described and provided by four experiments : liquid chromatography of papaverine and papaveraldine, dihydrogenophosphate determination with papaveraldine as IIR., liquid chromatography of PV_2 and benzoyl-PV_2 and 4-hydroxy-6 demethyl papaveraldine chromatography. An hypothetic structure of ion-pair is proposed.

INTRODUCTION

Ion-pair formation between isoquinoline derivatives and several inorganic anions was described in 1955 by GARDENT (1) and further applied to the purification of isoquinolines, by chloroform extraction of their salts (1). 1 - Benzylisoquinoline derivatives, whose leader in therapeutics is papaverine, exhibit the same property and thus enables the separation of papaverine from its main degradation product, papaveraldine, using ion pair liquid chromatography (2). However, we found that the presence of a phosphate buffer in the mobile phase induces abnormal retention of papaveraldine, as compared with papaverine. In order to explain this phenomenon, we developed a series of chromatographic experiments with benzoylisoquinolines and dihydrogenophosphate ions, showing the occurence of this abnormal retention. We provide here conclusive evidence of the greater stability of dihydrogenophosphate - 1 - benzoylisoquinoline ion pairs, and we propose a molecular structure explaining this feature.

EXPERIMENTAL

1. Reagents

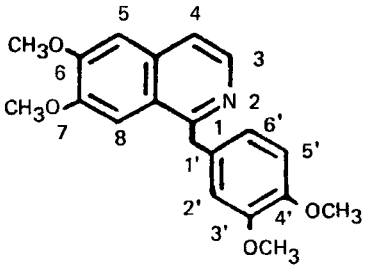
Papaverine was obtained from the Fluka Co (Buchs, SWITZERLAND). Papaveraldine (fig 1) was synthesized as described by BURGER (3) (oxidation of papaverine by

selenium dioxide) and was followed by toluene recristallisation. 4-(parachlorobenzyl)-6,7- dimethoxyisoquinoline (PV_2) (fig 1) was prepared according to the now classical method described by PRUDHOMMEAUX and BOUVIER (4,5). Derivatives, 4 para(benzoyl PV_2) and 4-hydroxy-6-demethyl papaveraldine (fig 1) were obtained by vanadic oxidation of, respectively, PV_2 (6) and papaverine (7).

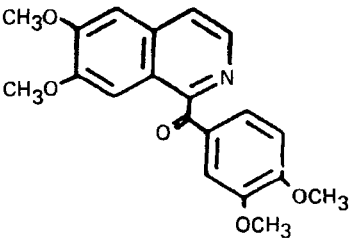
Sodic phenytoin and isoquinoline were obtained from the Fluka Co and tetrabutylammonium hydroxide (40 % solution) was supplied by the Sigma Co (Saint Louis, Ill., USA). Phosphate buffer was prepared using analytical grade phosphoric acid (Carlo Erba, Milano, Italy) and rectapur^R sodium dihydrogenophosphate (Prolabo, Paris, France). All solvents (Prolabo), were reagent grade. Water was deionized using high capacity ion exchangers.

2. Apparatus

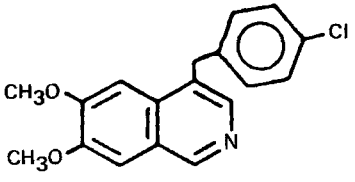
The HPLC system consisted of a chromatem 380 pump, a 7125 Rheodyne injector with a 20 µl sample loop, and either a SPD2A variable wavelength UV detector (Shimadzu, Kyoto, Japan) or a HP1040A diode assay UV detector (Hewlett-Packard, Fort Collins, USA) connected to an HP85A computer. The C_{18} reversed-phase column was a micro-Bondapak, (10 µm, 300 x 3.9 mm).



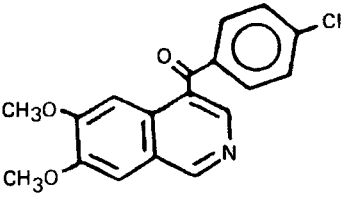
Papaverine



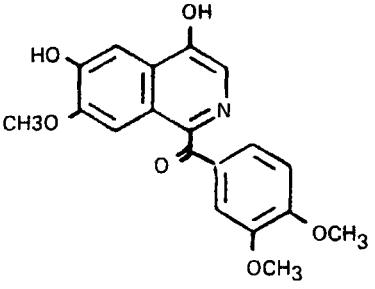
Papaveraldine



4- (parachiorobenzyl)6, 7-dimethoxy isoquioline (PV2)



4-(parachiorobenzoyl)6, 7-dimethoxy isoquinoline (benzoyl PV2)



4-hydroxy 6-demethyl papaveraldine

FIGURE 1

3. Chromatographic conditions

Four methods were tested :

N°1 : Separation of papaverine and papaveraldine, with a methanol - 0,1 M phosphate buffer pH 2.5 (50 - 50, v/v) mobile phase. The flow rate was of 1.5 ml/min, and the detection wavelength of 240 nm. Phenytoïn was used as internal standard. The results were compared with those obtained with the following eluent : 50 parts of methanol, 50 parts of an 0.01 M ammonium carbonate aqueous solution, and 2 parts of tetrahydrofuran, (approximately pH 7.9). Isoquinoline was used as internal standard, and the flow rate was of 1.6 ml/min.

N°2 : Analysis of inorganic anions (phosphate, chloride, bromide, iodide, nitrate) with an acetonitrile-0.01 M citrate buffer pH 3.0 (20 - 80, v/v) mobile phase, containing also 70 mg/l of papaveraldine perchlorate and 1.70 ml/l of a 40 % aqueous solution of tetrabutylammonium hydroxide. The flow rate was set at 1 ml/min and the detection wavelength at 360 nm.

N°3 : Determination of PV_2 and benzoyl- PV_2, with an acetonitrile - 0,1 M phosphate buffer pH 2.5 mobile phase. The internal standard was papaverine. The flow rate was 1 ml/min, and the detection wavelength set at 254 nm.

N° 4 : Separation of two tautomeric forms of 4-hydroxy 6-demethyl papaveraldine, using a methanol - 0.1 M phosphate buffer pH 3.5. The flow rate was 1.5 ml/min, and the multiwavelength detection ranged from 205 to 475 nm.

RESULTS AND DISCUSSION

Experiment N°1 : As papaveraldine is more polar than papaverine, it should elute first in a reversed phase system. Also, it is important to take into account the ionization of these molecules, as the pKa_s of the two bases are very close : pKa = 5.5 for papaverine and 4.6 for papaveraldine (2). Using the methanol- ammonium carbonate pH 7.9 mobile phase, the two bases are not protonated, and papaveraldine is actually eluted first. But when working with the methanol-phosphate buffer pH 2.5 mobile phase, the two bases are fully protonated, and the order of elution is inverted (fig 2). This unexpected retention of papaveraldine, may be explained by the presence of the dihydrogenophosphate ions (pKa_1 = 2.1), which give a more stable ion-pair with papaveraldine than with papaverine. This ion-pair, less polar than protonated papaverine, is better retained. Indeed, when replacing the phosphate buffer by an aqueous solution of chlorhydric acid pH 2.5, this phenomenon is not observed, and the order of

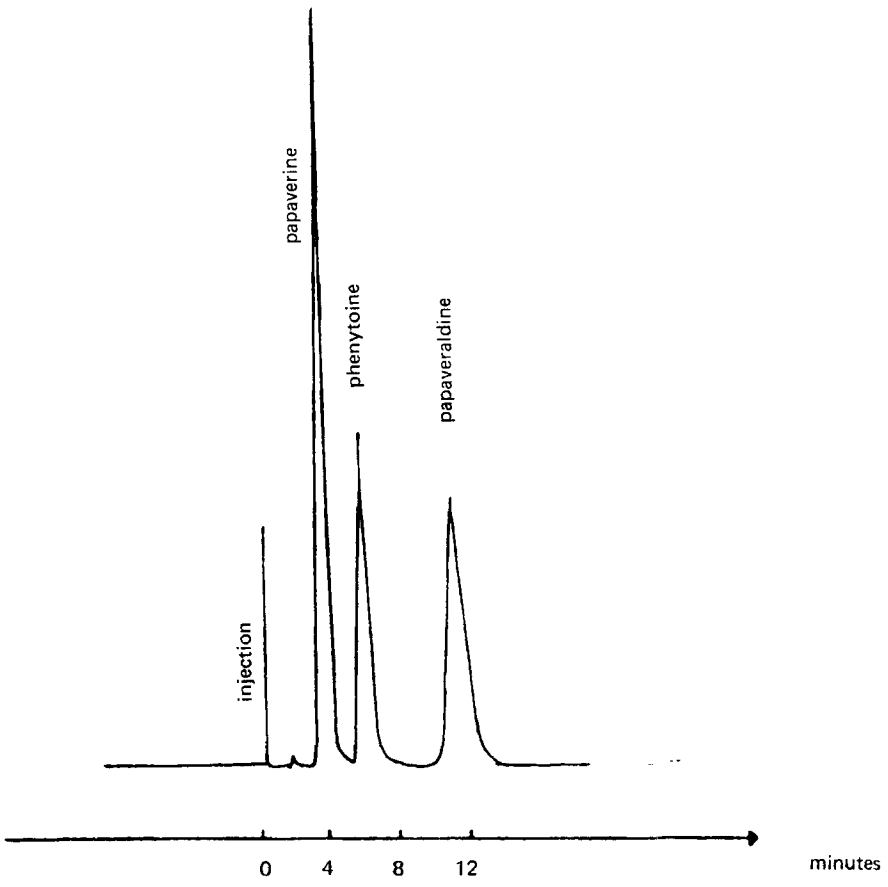


FIGURE 2

elution is the one expected. This exceptional stability of the dihydrogenophosphate-papaveraldine ion-pair was confirmed in the following experiment.

Experiment N°2 : Analysis of inorganic anions by ion-interaction chromatography is now a widely used method

(8).It consists in adsorbing an hydrophobic cation onto the stationary phase, which retains, by electrostatic interaction, the anions flowing in the mobile phase. Moreover, the presence in the mobile phase of either a cation or an anion showing UV-absorption, called IIR (Ion Interaction Reagent), results in UV detection of inorganic anions, even those lacking spectral properties. Whatever IIR used, the order of elution is always very close to the following list : F^-, Cl^-, NO_2^-, Br^-, I^-, SO_4^{2-}, PO_4^{3-} for the most common anions. We took papaveraldine as IIR, because to its properties : ion-pair formation and strong UV absorption. The results (fig 3) prove the value of

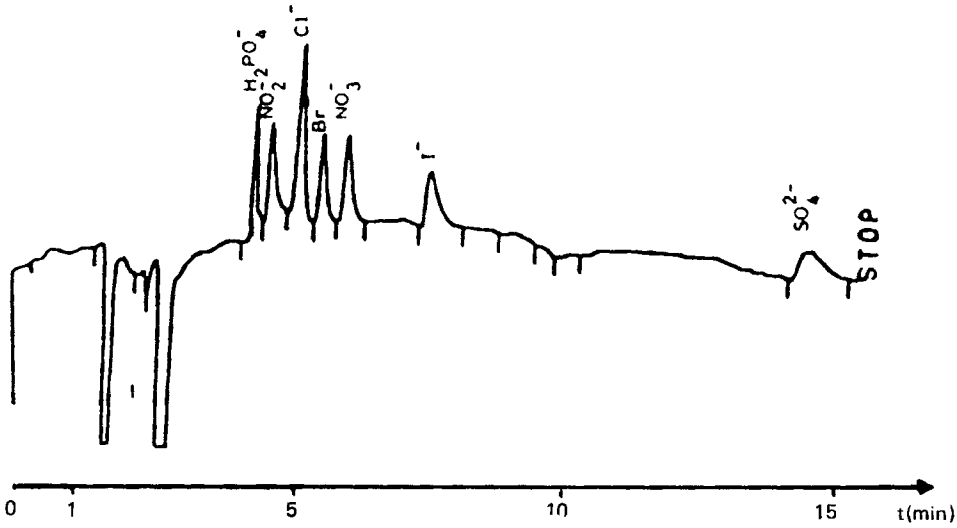


FIGURE 3

papaveraldine as IIR, the anions mentioned above being well resolved except for fluoride which is eluted in the solvent peak. However, the dihydrogenophosphate ions exhibit a surprisingly low retention, with regard to the usual elution order. This can be explained, as in the previous experiment, by a larger stability of the ion-pair papaveraldine-dihydrogenophosphate. Indeed, we have checked, by UV-determination, that papaveraldine is not retained by the column in our operating conditions. It is then found in the mobile phase, entirely ionized.Dihydrogenophosphate anions, combined with papaveraldine, are no longer retained by the tetrabutylammonium adsorbed onto the stationnary phase. The other anions, forming a less stable ion-pair with papaveraldine, are eluted in the usual order, with a stronger retention.

The great stability of the dihydrogenophosphate-papaveraldine ion-pair may be explained by the formation of a cyclic-structure stabilized by hydrogen and - ionizated bonds (# 8 cal), depicted in figure 4. In order to verify this assumption, ion-pair formation with other benzoylisoquinolines was implemented.

Experiment N°3 : Separation of PV_2 and benzoyl- PV_2 was not achieved with the mobile phase used in the first experiment (methanol - phosphate buffer pH 2.5,

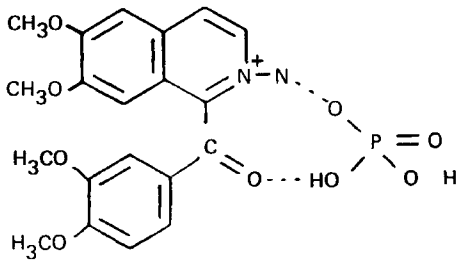


FIGURE 4

50-50, v/v)). Replacement in the same proportions of methanol by acetonitrile, which has a larger eluotropic strength, enabled complete separation of these two compounds (fig. 5). Theoretically, benzoyl-PV_2 is eluted first, as it is more polar than PV_2. In our experimental conditions, there was no inversion in the retention of the two products. This proves that the dihydrogenophosphate - benzoyl PV_2 ion-pair is not more stable than the dihydrogenophosphate - PV_2 one. This is logical when considering that the ketone function in 4 is unable to establish an hydrogen bond with the dihydrogenophosphate ion, as it is further away than in the case of papaveraldine.

Experiment N°4 : 4 - hydroxy 6-demethyl papaveraldine shows two pKa_S : pKa_1 = 4.07 and pKa_2 = 9.54. The former is due to the ionization of the isoquinoline's amine function, whereas the latter fits with the ioni-

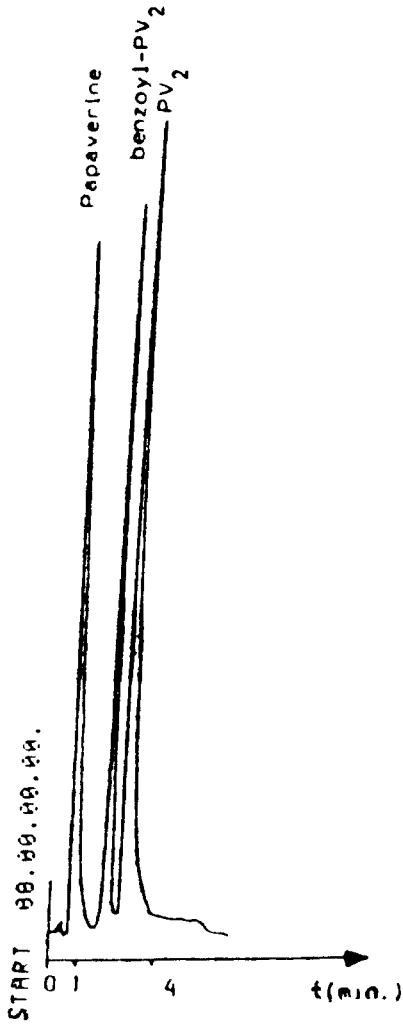


FIGURE 5

zation of the two phenolic functions (7). At pH 3.5 the basic and the protonated forms of the molecule coexist but only the protonated one is likely to form an ion-pair with dihydrogenophosphate. These structures being rather stable, the separation of the ion-pair from the 4-hydroxy 6-demethyl papaveraldine basic form is possible. In order to accurately identify these two species, we used a multiwavelenth absorbance detection mode. Figure 6 shows the resulting chromatogram. The basic form, green-coloured, was characterized by an absorption band with a maximum at 460 nm (t_R = 9.9 min) whereas the protonated form (ion pairing with phosphate), yellow-coloured, was characterized by an absorption band with a maximum at 400 nm (t_R = 2.8 min). The separation of these two forms seeming surprising, we performed chromatographies at pH 2,5 and 5,5 obtained for each structure a distinct peak at 2.8 and 9.9 min. The existence of these two peaks at pH 3,5 may thus be explained by the closeness of pKa, to this pH value and by the absence of an equilibrium between these two forms in the column. This experiment confirmed that the simultaneous presence of the protonated amine function and the ketone function in position 1 is essential for obtaining a stable ion-pair with dihydrogenophosphates ions. Mo-

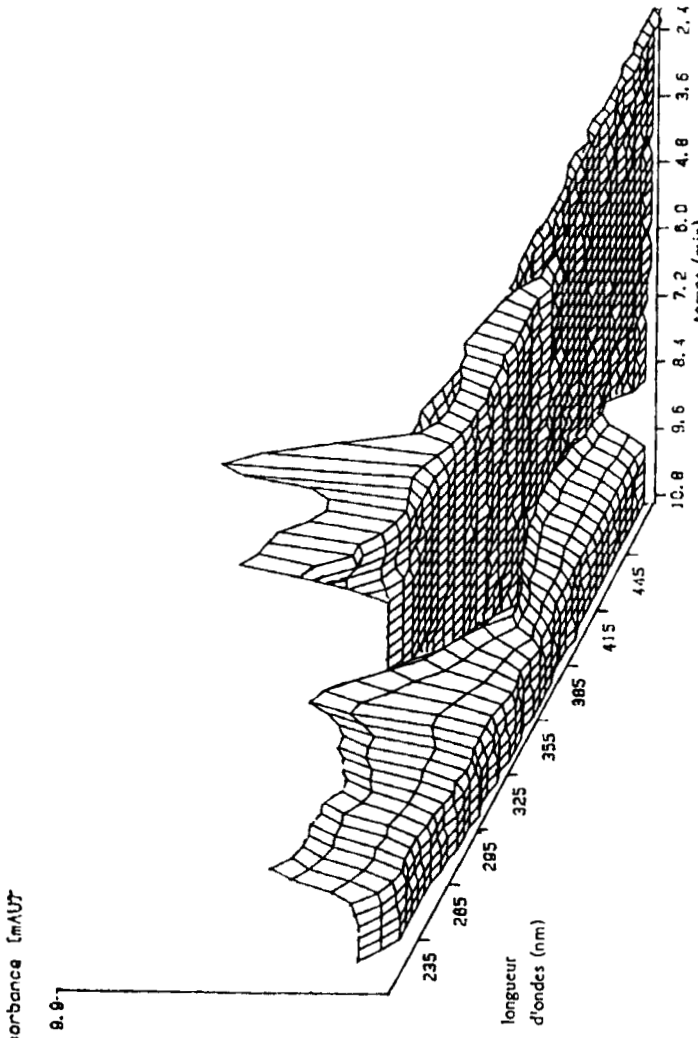


FIGURE 6

reover, it showed the possibility of achieving chromatographic separation of two tautomeric forms of a given molecule.

To sum up, papaveraldine and its derivatives are molecules able to form ion-pairs, the dihydrogenophosphate ones showing particular stability. This may be explained by the formation of a cyclic structure, stabilized by an hydrogen bond formed between an hydroxyl function of dihydrogenophosphate and the ketone function in 1. This assumption allows for better understanding of several abnormal retention phenomena that we previously mentioned in this paper. We are now investigation the usefullness of papaveraldine as IIR for the analysis of anion content of hemodialysis water.

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