CHROMSYMP. 1409

Note

Preparative liquid chromatographic separation of isomers of prostacyclin carba-analogues and their intermediates

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The carba-analogues of prostacyclin are its chemically stable and biologically active analogues[1] and a significant number of these compounds have been synthesized and tested for biological activity[2]. It is clear that every variation in their configuration causes a drastic change in their activity[3,4]. Moreover, in many of the synthetic stages the stereochemistry of these analogues is not completely controllable by chemical methods[5]. Therefore, problems arise in of the preparative separation of the mixtures of isomers formed.

In the synthesis of the carba-analogues of prostacyclin mainly three types of isomers can be distinguished, requiring chromatographic separation[5,6]: (1) regio isomers formed in the epoxide ring-opening reactions with various organometallic reagents; (2) *E/Z* geometric isomers formed by the Wittig olefination of ketones; and (3) α/β-stereoisomers at the C-15 position[5,6] of the prostacyclin molecule (in this instance chemical control over the isomer ratio is completely lacking).

Most of these isomers can be successfully resolved, but the separation of the C-15 α/β-stereoisomers of the 13,14-didehydro carba-analogues requires the formation of cobalt complexes prior to column chromatography (this lengthens the synthetic process)[7,8].

Among the factors determining the resolution and, consequently, the load-ability, throughput and cost in preparative separations, the selectivity of resolution (separation factor, α) is of primary importance[9,10]. Literature data on the selectivity of resolution of the above isomers are not available (mostly silica gel was used as the stationary phase, but different mobile phases have been used by different investigators). Therefore, we have studied the selectivity of resolution of these isomers. We also show that this approach has been successful in the semi-preparative separation of the C-15 α/β-isomers of a new 13,14-didehydro carba-analogue of prostacyclin without the use of cobalt complexes.

EXPERIMENTAL

All the compounds studied were synthesized at the Institute of Chemistry of the Academy of Sciences of the Estonian S.S.R. (see Fig. 1). Their chemical identities were
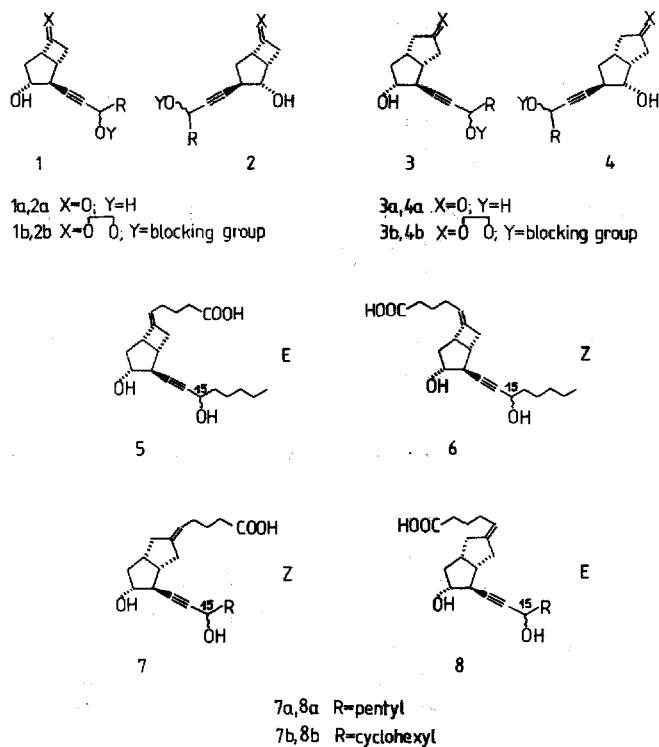
1a,2a X=O; Y=H
1b,2b X=O̅ O̅; Y=blocking group

3a,4a .X=O; Y=H
3b,4b X=O̅ O̅; Y=blocking group

7a,8a R=pentyl
7b,8b R=cyclohexyl

Fig. 1. Formulae of the compounds studied.

established by ^{13}C NMR spectroscopy. Separations were performed on a DuPont Model 8845 liquid chromatograph, equipped with UV spectrophotometric and refractometric detectors.

Separation factor measurements were performed with a Zorbax SIL (DuPont) analytical column (150 mm × 4.6 mm I.D.). The semi-preparative resolution of compounds 7b and 8b was performed on two Zorbax SIL Golden Series columns (80 mm × 6.2 mm I.D.) coupled in series. Their total plate number was 12 500 at a flow-rate of 1.2 ml/min, as calculated by using the naphthalene peak.

All the solvents used were purchased from Reakhim (Moscow, U.S.S.R.). Their preparation before use is described in ref. 11.

RESULTS AND DISCUSSION

The resolution of the regio isomers formed is usually performed at the stage of blocked (1a–4a) or unblocked (1b–4b) ketonediols. The selectivity of their resolution on silica gel has been reported[12]. It has been shown that the separation factor, α, varies significantly depending on the localization parameters m and m^0 and on the hydrogen bonding between solutes and mobile phases. It is essential to note that a selectivity of resolution of up to $\alpha = 2.0$ can be achieved. This means that only 100 theoretical plates

are necessary for their complete resolution, and sample loads of up to 10 mg per gram of adsorbent (50–100 μm) can be applied[10].

The selectivity of resolution of the E/Z isomers of prostacyclin carba-analogues has been reported previously[13]. It has been shown that the selectivity depends greatly on the structure of the isomers. In one instance it exceeded 2.0 on silica gel, but often low α values are encountered[13]. Therefore, we examined the solvent selectivity on the example of compounds 5 and 6 (the most difficult to resolve pair of E/Z isomers) using binary mobile phases A–B (modified with water at concentrations up to saturation in order to avoid peak tailing and loss of efficiency of resolution[11]), where A is, n-hexane, benzene or chloroform and B is 2-propanol, ethanol, methanol, acetonitrile, acetone or ethyl acetate. The α value in the solvent systems studied varied from 1.08 to 1.19, thus increasing the loadability significantly and resulting in savings on scaling up the resolution (data for some mobile phases yielding higher selectivities of resolution are presented in Table I).

TABLE I

CAPACITY FACTORS (k') AND SELECTIVITY OF RESOLUTION (α) OF COMPOUNDS 5 AND 6

Column, Zorbax SIL (150 mm × 4.6 mm I.D.); temperature, 35°C; flow-rate, 0.6 ml/min.

Mobile phase	k'		α
	Compound 5	Compound 6	
Benzene–2-propanol–water (97:2.95:0.05)	8.30	9.88	1.19
Chloroform–2-propanol–water:			
95:4.94:0.06	4.20	4.90	1.18
96:3.94:0.06	7.43	8.56	1.18
Chloroform–ethanol–water:			
96:3.83:0.17	4.32	4.94	1.15
97:2.87:0.13	8.98	10.5	1.18
n-Hexane–2-propanol–water			
96:3.86:0.14	5.37; 5.50	6.03; 6.22	1.10
97:2.9:0.1	18.3; 18.6	21.4; 22.0	1.14

However, as shown in Table I, only with n-hexane–2-propanol as the mobile phase can resolution of the C-15 α/β-isomers of 13,14-didehydro analogues be achieved. The four-membered ring carba-analogues 5 and 6 were not identified because the resolution is still insufficient for their preparative resolution. However, as shown previously[13], the five-membered ring carba-analogues 7a and 8a can be resolved with a resolution of $R_s = 1.0$ on a column having 9700 theoretical plates. In this study, we resolved semi-preparatively the C-15 α/β-isomers of carba-analogues 7b and 8b (Fig. 2). Thus, 24 mg of crude synthetic mixture (previously filtered through silica gel) afforded, on eight injections, 2–4 mg of each of the four isomers of the new prostacyclin carba-analogues 7b and 8b in pure form and allowed the determination of their biological activities. It should be noted that this is a case where the resolution of E- and Z-isomers also requires the use of high-performance liquid chromatography
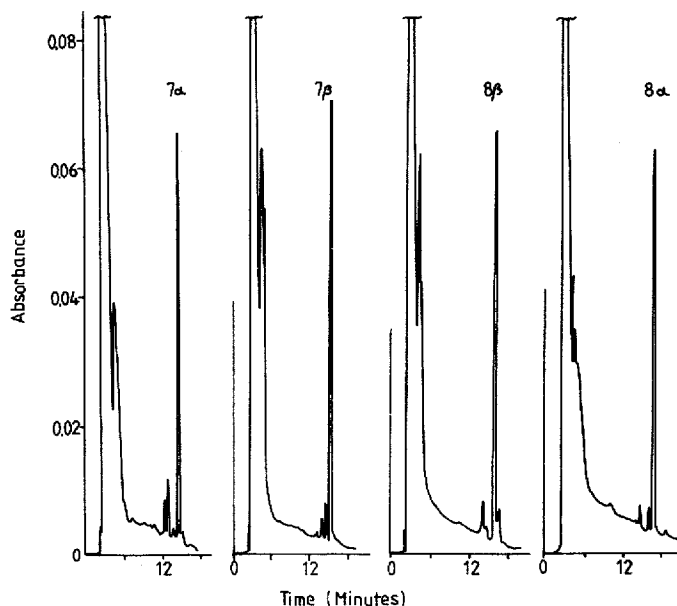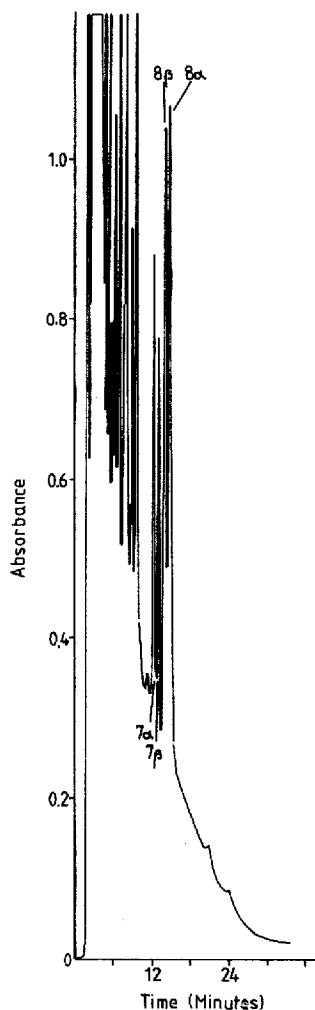
Fig. 2. Semi-preparative resolution of the C-15 α/β-isomers of compounds 7b and 8b. Columns, two Zorbax SIL Golden Series columns (80 mm × 6.2 mm I.D.) in series; mobile phase, *n*-hexane–2-propanol–water (90:9.9:0.1); flow-rate, 1.2 ml/min; detection, 210 nm; sample size, 3 mg injected in 50 μl of benzene. α and β refer to the corresponding C-15 isomers.

Fig. 3. Purity of the C-15 α/β-isomers of compounds 7b and 8b. Sample size, 25 μg injected in 5 μl of benzene. Other chromatographic conditions as in Fig. 2.

(HPLC). The isomers were checked for purity on the same columns under analytical conditions (Fig. 3). It is apparent that the purity of throughput could easily be increased by using a column (or columns of nearly equal performance connected in series) having up to 20 000 theoretical plates. For this purpose, a column (or columns) packed with 5-μm particles may be used.

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