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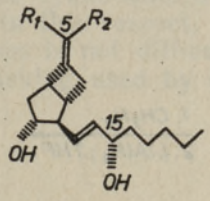
POSSIBILITIES OF SEPARATING 5*E*/*Z* AND 15α/β ISOMERS OF PROSTACYCLIN CARBA-ANALOGS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

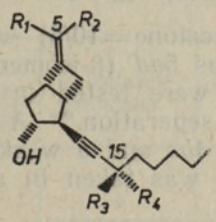
The carba-analogs of prostacyclin (PGI_2) have been stated chemically stable and biologically highly potent substances [1, 2]. Every variation in their configuration (e. g., at C5 and C15, prostaglandin numeration) causes a drastic change in their activity [2].

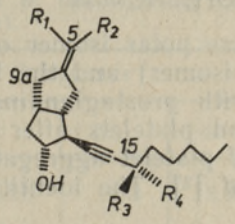
In the synthesis of racemic prostacyclin carba-analogs the configuration at C15 is usually not controlled by a synthetic method. So, the problem of separating 15α/β-isomers arises [3, 4]. Moreover, the final step of synthesis, the α-chain formation by the Wittig olefination, is not stereoselective and leads to an *E*/*Z* mixture at C5 of these compounds [5, 6]. Therefore we have studied methods of separating 5*E*/*Z* and 15α/β-isomers of compounds 1—4 * that may be used on a preparative scale.



1*a*. R_1=H; R_2=$(CH_2)_3COOH$
1*b*. R_1=$(CH_2)_3COOH$; R_2=H

2*a*. R_1=H; R_2=$(CH_2)_5COOH$
2*b*. R_1=$(CH_2)_5COOH$; R_2=H





3*ac*. R_1=R_3=H; R_2=$(CH_2)_3COOH$; R_4=OH
3*ad*. R_1=R_4=H; R_2=$(CH_2)_3COOH$; R_3=OH
3*bc*. R_1=$(CH_2)_3COOH$; R_2=R_3=H; R_4=OH
3*bd*. R_1=$(CH_2)_3COOH$; R_2=R_4=H; R_3=OH

4*ac*. R_1=R_3=H; R_2=$(CH_2)_3COOH$; R_4=OH
4*ad*. R_1=R_4=H; R_2=$(CH_2)_3COOH$; R_3=OH
4*bc*. R_1=$(CH_2)_3COOH$; R_2=R_3=H; R_4=OH
4*bd*. R_1=$(CH_2)_3COOH$; R_2=R_4=H; R_3=OH

* The letter *a* at compound number denotes a *prostaglandin-like* structure of carbacyclin analogs (see formulas above) and the letter *b* at compound number denotes a *prostacyclin-like* structure of prostacyclin carba-analogs. The letter *c* denotes the 15α-isomery of compounds, and *d* the 15β-isomery.

L. Floche et al. [2] have used a reversed-phase mode of chromatography to separate some of the 5*E*/*Z*-isomers of prostacyclin carba-analogs, but the method proposed by them requires troublesome extraction of the product from the water-containing eluting mixture, and therefore it should not be used for preparative separation. We have chosen a liquid-solid mode of chromatography over silica gel and a normal-phase mode of bonded-phase chromatography over nitrile-modified packing, using easily removable hexane-isopropanol mixtures for elution.

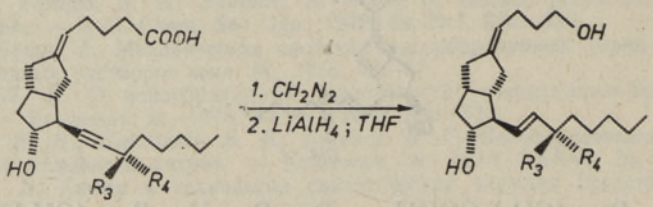
Materials and methods

Apparatus. A DuPont HPLC system N 8845 with the UV spectrophotometrical detector was used.

Columns. Zorbax SIL 4.6×150 mm, initially 9700 theoretical plates and Zorbax CN 4.6×250 mm, initially 16 200 theoretical plates, were used.

Solvents. Hexane (Reakhim) was used without purification; isopropanol (Reakhim) was rectified, subsequently its water content was adjusted to 3.5%.

Samples. Compounds 1—4 were synthesized by the authors of the present work and identified by ^{13}C NMR spectroscopy at the Institute of Chemical Physics and Biophysics, Tallinn.* To identify 4*ac* and 4*ad*, the compounds were subjected to preparative separation, methylation with diazomethane and reduction with $LiAlH_4$ to yield easily separable olefinic prostanoids 5*ac* and 5*ad*.



4*ac*. R_3=H; R_4=OH
4*ad*. R_3=OH; R_4=H

5*ac*. R_3=H; R_4=OH
5*ad*. R_3=OH; R_4=H

The more polar isomer on TLC (benzene/acetone=10/6) was taken as 5*ac* (α-isomer) and the less polar isomer as 5*ad* (β-isomer) by the analogy with prostaglandins. Compounds 4*b* were tested *in vitro* on rabbit blood platelets after their preparative separation.** A stronger inhibitor of platelet aggregation was taken as 4*bc* and a weaker inhibitor as 4*bd* [2]. The identity of compounds 3 was taken in a similar manner.

Chromatographic conditions. Hexane and isopropanol in different ratios were used as a mobile phase. Experiments were performed at 35 °C and at a flow rate of 0.6 ml/min, and detection was performed at 210 nm.

Calculations. The capacity factors (k') were calculated according to the formula

$$k' = \frac{t_R - t_0}{t_0},$$

where t_R — retention time (measured from the chromatogram, mm), t_0 — retention time of unretained compound (mm). t_0 was calculated

* The authors are indebted to T. Välimäe for ^{13}C NMR investigations.
** The authors are indebted to A. Lopp for platelet aggregation tests.

from the operational values of the void volume of column (V_0), chart speed and flow rate. V_0 was determined as an elution volume of toluene using a 75/25 hexane/isopropanol mixture as a mobile phase. V_0 for Zorbax SIL was found to be 1.89 ml and for Zorbax CN 3.00 ml.

The resolution factors (α) were calculated according to the formulas:

$$\alpha = k'_b / k'_a \quad \text{(for } 5E/Z\text{-isomers)},$$

$$\alpha = k'_c / k'_d \quad \text{(for } 15\alpha/\beta\text{-isomers)},$$

where k'_b, k'_a, k'_c, k'_d are capacity factors of the respective isomers *b*, *a*, *c*, and *d* of compounds 1—4.

The resolution function (R_s) was estimated according to [7].

Results and discussion

The results obtained in the resolution of *E*/*Z*-isomers of compounds 1—4 on Zorbax SIL and Zorbax CN are listed in Tables 1 and 2, respectively. It is evident that silica gel offers better selectivity for the resolution of *E*/*Z*-isomers than a cyano-bonded phase, but in the latter case the selectivity of the resolution (see Fig. 1*A* and 1*B*) is sufficient, too (except for compound 3). It is noteworthy that the silica gel columns are to be deactivated with water to avoid peak tailing before the resolution of carboxylic acids [7] that leads to serious problems in preparing the columns. The cyano-bonded phase columns are superior to the silica gel columns in this respect, and can be used successfully when the resolution problem is not difficult. In the case of the mobile phase and the types of packing used by the authors, the selectivity of

Table 1

Dependence of the resolution factor (α) and resolution function (R_s) of *E*- and *Z*-isomers of compounds 1—4 and the capacity factor (k'_b) of compounds 1*b*, 2*b*, 3*bc*, 3*bd*, 4*bc* and 4*bd* on the isopropanol content in hexane using column Zorbax SIL (4.6×150 mm).

Isopropanol content in hexane, %	Calculated parameter	Compounds					
		1	2	3*c*	3*d*	4*c*	4*d*
4	k'_b	8.66	6.07	6.22	6.03	5.96	5.65
	α	1.29	1.64	1.16	1.10	1.40	1.25
	R_s	large	large	large	1.0	large	large
3	k'_b	34.8	22.6	22.0	21.4	18.9	17.8
	α	1.42	2.07	1.21	1.14	1.59	1.40
	R_s	large	large	large	1.25	large	large

Table 2

Dependence of the resolution factor (α) and resolution function (R_s) of *E*- and *Z*-isomers of compounds 1—4 and the capacity factor (k'_b) of compounds 1*b*, 2*b*, 3*bc*, 3*bd*, 4*bc* and 4*bd* on the isopropanol content in hexane using column Zorbax CN (4.6×250 mm).

Isopropanol content in hexane, %	Calculated parameter	Compounds					
		1	2	3*c*	3*d*	4*c*	4*d*
4	k'_b			2.57	2.57	2.24	2.24
	α			1.06	1.06	1.19	1.13
	R_s			0.8	0.8	1.25	1.0
3	k'_b	4.97	3.69	5.72	5.72	5.49	5.49
	α	1.18	1.71	1.08	1.08	1.26	1.20
	R_s	1.25	large	0.9	0.9	large	1.25
2	k'_b	9.76	6.64	10.7	10.7	8.44	8.44
	α	1.27	2.00	1.12	1.12	1.36	1.29
	R_s	large	large	1.0	1.0	large	large

Table 3

Dependence of the resolution factor (α) and resolution function (R_s) of 15α/β-isomers of compounds 3 and 4 and capacity factor (k'_b) of compounds 3*ac*, 3*bc*, 4*ac*, 4*bc* on the percentage of isopropanol in hexane using column Zorbax SIL (4.6×150 mm).

Isopropanol content in hexane, %	Calculated parameter	Compounds			
		3*a*	3*b*	4*a*	4*b*
4	k'_c	5.37	6.22	4.25	5.96
	α	0.98	1.03	0.94	1.06
	R_s	0.5	0.6	1.0	1.0
3	k'_c	18.3	22.0	11.9	18.9
	α	0.97	1.03	0.93	1.06
	R_s	0.6	0.65	1.0	1.0

resolution can be improved by reducing the isopropanol content in hexane. In comparison with the reverse-phase mode chromatography [8], the normal-phase mode of chromatography gives higher α-values for the resolution of *E*/*Z*-isomers of prostacyclin carba-analogs. That means that sufficient separation of these isomers may often be distinguished

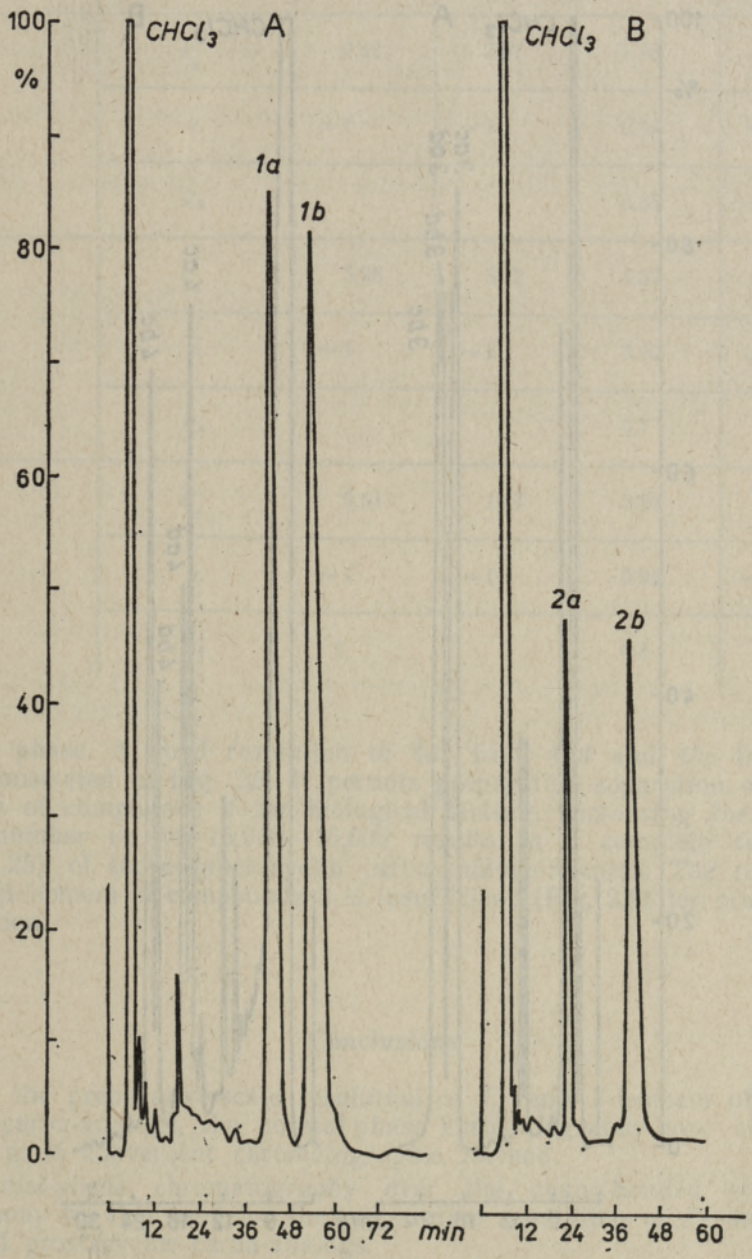


Fig. 1. Chromatograms of separation of compounds 1 (*A*) and 2 (*B*). Conditions: Column Zorbax CN (4.6×250 mm); mobile phase — hexane/isopropanol = 98/2; flow rate — 0.6 ml/min; column temperature — 35 °C; detection — UV 210 nm; absorbance — 0.16 AUFS; chart speed — 5 cm/h; sample size — ~100 μg (*A*) and ~50 μg (*B*).

using columns with a lower plate number, e. g. by conventional liquid chromatography over silica gel or over cyano-bonded stationary phase.

Concerning the resolution of 15α/β-isomers of compounds 3 and 4 columns Zorbax SIL and Zorbax CN (Tables 3 and 4, respectively) it is evident that silica gel has also a higher selectivity than the cyano-

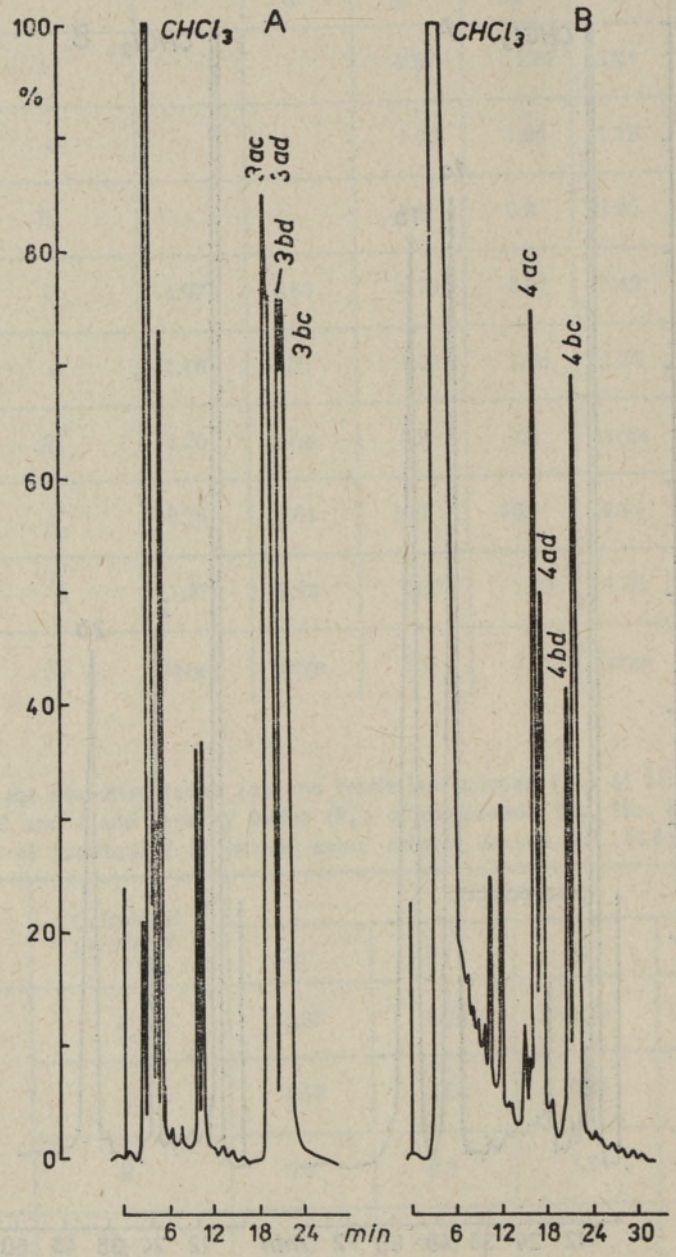


Fig. 2. Chromatograms of separation of compounds 3 (*A*) and 4 (*B*). Conditions: Column — Zorbax SIL (4.6×150 mm); mobile phase — hexane/isopropanol = 96/4; flow rate — 0.6 ml/min; column temperature — 35 °C; detection — UV 210 nm; absorbance — 0.16 AUFS; chart speed — 10 cm/h; sample size — ~50 μg (*A*) and ~40 μg (*B*).

Table 4

Dependence of resolution factor (α) and resolution function (R_s) of 15α/β-isomers of compounds 3 and 4 and capacity factor (k'_c) of compounds 3*ac*, 3*bc*, 4*ac* and 4*bc* on the percentage of isopropanol in hexane using column Zorbax CN (4.6×250 mm).

Percentage of isopropanol in hexane	Calculated parameter	Compounds			
		3*a*	3*b*	4*a*	4*b*
4	k'_c	2.43	2.57	1.88	2.24
	α	~1	~1	0.95	~1
	R_s			0.65	
3	k'_c	5.28	5.72	4.37	5.49
	α	~1	~1	0.95	~1
	R_s			0.7	
2	k'_c	9.61	10.7	6.20	8.44
	α	~1	~1	0.94	~1
	R_s			0.8	

bonded phase. A good resolution of 4*ac* from 4*ad* and 4*bc* from 4*bd* is demonstrated in Fig. 2*B*. It permits preparative separation of 15α/β-isomers of compounds 4 for biological testing. Increasing the column plate number up to 15,000—16,000 results in a complete resolution ($R_s \geqslant 1.25$) of those prostacyclin carba-analogs-isomers. The resolution of 15α/β-isomers of compounds 3 is insufficient (Fig. 2*A*) for preparative separation.

Conclusions

1. For the preparative-scale resolution of *E*- and *Z*-isomers of prostacyclin carba-analogs, the normal-phase chromatography over silica gel is the most convenient chromatographic method.

2. Normal-phase chromatography over the cyano-bonded stationary phase may be also used for the preparative separation of *E*- and *Z*-isomers of prostacyclin carba-analogs.

3. The semipreparative resolution of 15α- and 15β-isomers of 5*E*- and 5*Z*-9-desoxy-13,14-didehydro-6,9α-methano-Δ^5-PGF_1 was achieved using the chromatographic method over silica gel.

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PROSTATSÜKLIINI KARBA-ANALOOGIDE *5E/Z*- JA 15α/β-ISOMEERIDE PREPARATIIVSE LAHUTAMISE VÕIMALUSED KÕRGEFEKTIIVSE VEDELIKUKROMATOGRAAFIA ABIL

On uuritud prostatsükliini nelja erineva karba-analoogi 5*E*- ja 5*Z*-isomeeride ning kahe analoogi 15α- ja 15β-isomeeride preparatiivse lahutamise võimalusi normaalfaasilisel kõrgefektiivsel vedelikukromatograafiameetodil. Leiti, et silikageelkolonn omab suuremat selektiivsust uuritud isomeeride lahutamiseks kui nitriilmodifitseeritud kolonn. Kromatograafiliselt õnnestus preparatiivselt lahutada 5*E*- ja 5*Z*-9-desoksü-13,14-didehüdro-6,9α-metano-Δ^5-PGF_1 15α- ja 15β-isomeerid.

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ВОЗМОЖНОСТИ ПРЕПАРАТИВНОГО РАЗДЕЛЕНИЯ 5*E/Z*- и 15α/β-ИЗОМЕРОВ КАРБА-АНАЛОГОВ ПРОСТАЦИКЛИНА ПРИ ПОМОЩИ ВЫСОКОЭФФЕКТИВНОЙ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ

Изучались возможности препаративного разделения 5*E*- и 5*Z*-изомеров четырех карба-аналогов простациклина при помощи нормально-фазной высокоэффективной жидкостной хроматографии. Найдено, что силикагелевая насадка обеспечивает лучшую селективность разделения изученных изомеров, чем насадка, модифицированная нитрильной группировкой. Хроматографически удалось препаративно разделить также 15α- и 15β-изомеры 5*E*- и 5*Z*-9-дезокси-13,14-дидегидро-6,9α-метано-Δ^5-$ПГF_1$.