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HPLC RESOLUTION OF THE DIASTEREOISOMERS OF SULPHOXIDES FORMED BY OXIDATION OF DITHIOKETALES OF BICYCLO[3.2.0]HEPT-2-EN-6-ONE, ITS EPOXIDE, AND BROMOHYDRINE

The primary requirement the chiral biologically active compounds must meet is their enantiomeric purity. Among enantiomerically pure (e. p.) prostaglandin intermediates, bicyclo[3.2.0]hept-2-en-6-one, its epoxide, and bromohydrine are well-known [1, 2]. Chemical and biotechnological methods have been proposed for the preparation of e. p. parent ketone [1, 3, 4]. In the course of our search for alternative routes to e. p. bicycloheptenone derivates [5] the transformation of ketone to dithioketales with subsequent oxidation to chiral sulphoxides using the modified Sharpless reagent was examined [6, 7].

In the present work, HPLC resolution of the diastereomeric sulphoxides formed by the oxidation of dithioketales of ketone (1), its bromohydrine (2), and epoxide (3) by a chiral oxaziridine derivative [8] is investigated. The oxidation yields four diastereomers (Fig. 1). The separation of these diastereomers (on both analytical and preparative scale) is complicated due to the presence of unreacted reagents, a reduced oxidant, and side products in the reaction mixture. This paper reports the results obtained.

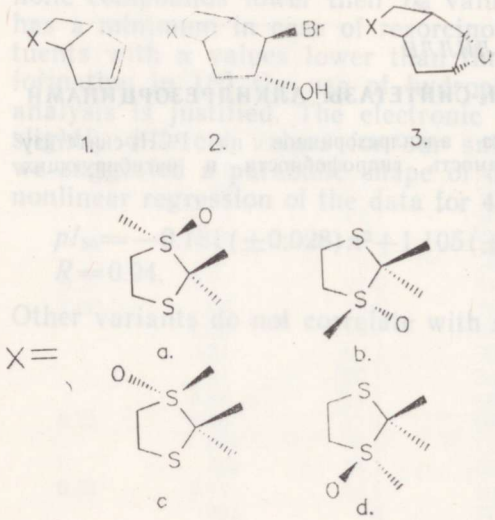

Fig. 1. The structure of diastereomers of sulphoxides of heptenone (1), bromohydrine (2), and epoxide (3) studied.

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Experimental

Apparatus. A Du Point HPLC system No. 8845 with a UV-spectrophotometrical detector was used for analytical and semipreparative separations. For these purposes Zorbax SIL columns (250×4.6 mm I. D. and 250×9.4 mm I. D.) with a theoretical plate number of about 15 000—18 000 were used. The eluent flow rates were 0.8 and 3.5 ml/min for the analytical and semipreparative columns, respectively. The columns temperature was 35 °C.

Preparative separation was carried out using a preparative HPLC system PVK-31A, Special Designing Bureau of the Estonian Academy of Sciences. The column (300×30 mm I. D.) was packed with 5 μm Separon SGX. The number of theoretical plates was approximately 5000. Separations were performed at 35 °C. The flow rate was 35 ml/min.

The UV-wavelength was chosen according to the transparency of the mobile phase system used. It was 225 nm for the *n*-hexane-isopropanol system, 254 nm for the chloroform-based system, 260 nm for the ethyl acetate containing systems, and 265 nm for the carbon tetrachloride-isopropanol-water one.

Solvents. All the solvents (analytical grade) were purchased from Reakhim, USSR. *n*-Hexane was used without purification. Chloroform, isopropanol, and carbon tetrachloride were redistilled. Chloroform free of a proton-donor stabilizer, ethanol, was prepared by washing it thrice with an equal amount of water, drying overnight with $CaCl_2$, and redistilling over P_2O_5. Ethyl acetate was redistilled over P_2O_5. Acetonitrile was rectified. Bidistilled water was used as a mobile phase modifier.

Samples. All the samples were synthesized and their structure was proved as described in [9]. For analytical measurements a 10 μl aliqout of the reaction mixture was taken and completely dissolved in 40 μl of benzene-ethyl acetate (3:1); 5—20 μl was injected. In the first step of preparative purification the reaction mixture was dissolved using dichloromethane (freshly distilled) and thereafter injected into the preparative column. After further purification various solvent mixtures proved to be useful for dissolving the sample. When polar solvents were used, the samples were diluted with benzene or chloroform in order to avoid the peak band broadening because of a high polarity of the solvent.

Calculations. The capacity and resolution factors were calculated as previously described [10]. The void volume of the analytical column was measured to be 3.41 ml [11].

Results and Discussion

To describe the chromatographic behaviour of different sulphoxides (Fig. 1) the capacity and resolution factors (Tables 1—4) of these compounds are used. In our previous study [7] the mobile phase consisting of carbon tetrachloride-methanol was used for the separation of heptenones. In this work, however, when carbon tetrachloride-isopropanol-water, the mobile phase with nearly comparable selectivity (mobile phase 9; Tables 1 and 2), was used the degradation of sulphoxides took place so that the carbon tetrachloride-based mobile phases were discarded. The chloroform-based mobile phases (6 and 8; Tables 1 and 2)

gave poor resolution of 1b and 1c. At the same time the selectivity with *n*-hexane-isopropanol-water mixtures (mobile phases 1—3) is low for 1a and 1b. 1d was not separated and identified. The formation of a quaternary solvent system *n*-hexane-chloroform-isopropanol-water (mobile phase 7) gives good compromise. The selectivity of *n*-hexane-ethyl acetate-water mixtures (mobile phases 4 and 5) may be assessed as satisfactory.

Considering these findings the chromatographic separation of heptenones (1) was carried out as follows: for the measurement of a diastereomeric ratio mobile phases 1 (see Fig. 2) and 3 were used. The detection at 225 nm provided high sensitivity. The somewhat low α value for 1a/1b is not critical for the analytical column. Oxaziridine and its reduced form are more retentive (their capacity factors in System 1 are 10.2 and 9.0, respectively) and their elution does not interfere with

Table 1

Capacity factors (k') of heptenones, bromohydrines, and epoxides

Column: Zorbax SIL (4.6×250 mm), V_0=3.41 ml. Temperature 35 °C

Mobile phase No.	Mobile phase composition V/V/V	Heptenones*		
		1a	1b	1c
	n-Hex/iPrOH/H_2O			
1.	85/14.98/0.02	3.78	4.11	5.80
2.	85/14.4/0.6	2.35	2.73	3.47
3.	90/9.6/0.4	3.28	3.9	5.02
	n-Hex/EtOAc/H_2O			
4.	20/79.2/0.8	2.03	2.33	2.96
5.	40/59.4/0.6	3.30	3.85	4.90
6.	$CHCl_3$ 100%	2.35	3.03	3.14
	n-Hex/$CHCl_3$/iPrOH (0.1% H_2O)			
7.	43/56/1	3.44	4.20	4.82
	$CHCl_3$/CH_3CN/H_2O			
8.**	94/5.76/0.24	2.30	3.04	3.20
	CCl_4/iPrOH/H_2O			
9.	97/2.95/0.05	3.74	4.61	6.18
		Bromohydrines		
		2a	2b	2c	2d
	n-Hex/iPrOH/H_2O				
10.	75/24/1	3.73	2.63	3.24	3.59
	n-Hex/EtOAc/H_2O				
11.	10/89.1/0.9	1.83	2.01	1.94	4.75
	n-Hex/EtOAc/iPOH/H_2O				
12.	68/9.9/21.1/1.0	2.90	2.38	2.60	3.32
	$CHCl_3$/iPrOH/H_2O				
13.	93/6.93/0.07	1.73	1.09	1.98	3.85
		Epoxides			
		3a	3b	3c	3d
	n-Hex/iPrOH/H_2O				
14.	60/38.4/1.6	3.79	4.73	6.41	3.51
	n-Hex/EtOAc/H_2O				
11.	10/89.1/0.9	4.54	5.71	5.71	4.35
	$CHCl_3$/iPrOH/H_2O				
15.	96/3.96/0.04	1.98	3.28	2.50	2.17

* Only three diastereomers are identified and separated.
** Specially purified chloroform was used (free of stabilizer).

Table 2

Resolution factors (α) of heptenones

Column: Zorbax SIL. Temperature 35 °C

Mobile phase No.*	Resolution factors, α		
	1b/1a	1c/1b	1c/1a
1.	1.09	1.41	1.53
2.	1.16	1.27	1.48
3.	1.19	1.28	1.53
4.	1.15	1.27	1.46
5.	1.17	1.27	1.48
6.	1.29	1.04	1.34
7.	1.22	1.15	1.40
8.	1.32	1.05	1.39
9.	1.23	1.34	1.65

* Mobile phase composition see Table 1.

Table 3

Resolution factors (α) of bromohydrines

Column: Zorbax SIL. Temperature 35 °C

Mobile phase No.*	Resolution factors, α					
	2b/2a	2c/2a	2c/2b	2d/2a	2d/2b	2d/2c
10.	0.71	0.87	1.23	0.96	1.37	1.11
11.	1.09	1.06	0.97	2.60	2.36	2.45
12.	0.82	0.90	1.09	1.15	1.40	1.27
13.	0.63	1.15	1.82	2.23	3.53	1.94

* Mobile phase composition see Table 1.

Table 4

Resolution factors (α) of epoxides

Column: Zorbax SIL. Temperature 35 °C

Mobile phase No.*	Resolution factors, α					
	3b/3a	3c/3a	3c/3b	3d/3a	3d/3b	3d/3c
14.	1.25	1.69	1.36	0.93	0.74	0.55
11.	1.26	1.26	1.00	0.96	0.76	0.77
15.	1.66	1.26	0.76	1.1	0,66	0.87

* Mobile phase composition see Table 1.

the resolution of the compounds of interest. However, the case is not so under preparative loadings, when the *n*-hexane-isopropanol-water mixture does not give oxidant-free sulphoxides 1. In the first-step preparative chromatography of heptenones, the mobile phase with a high selectivity for the separation of reagents must be used. For this purpose ethanolic chloroform or its mixture with a small amount of isopropanol is the most advantageous. The oxaziridine products are eluted nearly at a column void volume, as is the oxidation precursor, dithioketale (precursors of all sulphoxides, 1, 2, and 3 elute near column void volume in all the solvent systems investigated). In the course of this purification step, the enriched fractions of 1a, 1b, and 1c are also obtained. Their final purification is carried out semipreparatively using mobile phases 2, 5, or 7.

The oxidation of the corresponding bromohydrinic precursor with oxaziridine yields four diastereomeric sulphoxides 2. For the oxidation products profiling the UV-transparent mobile phase *n*-hexane-isopropanol-water is not suitable because of a very poor resolution of isomers 2a and 2d (see mobile phase 10, Table 3). On the other hand, by using *n*-hexane-ethyl acetate-water (mobile phase 11), isomers 2a, 2b, and 2c remain practically unresolved. So, a comparison of the retention

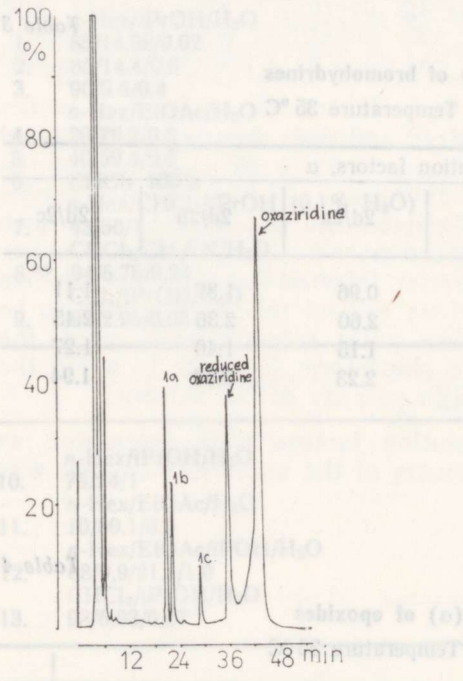

Fig. 2. A chromatogram of the separation of heptenones (1). Conditions: column Zorbax SIL (4.6×250 mm); mobile phase: *n*-hexane-isopropanol-water = 85 : 14.98 : 0.02; flow rate 0.8 ml/min; column temperature 35 °C; detection UV 225 nm; absorbance 0.32 AUFS; sample size: the reaction mixture corresponding to 50 μg of the initial dithioketale.

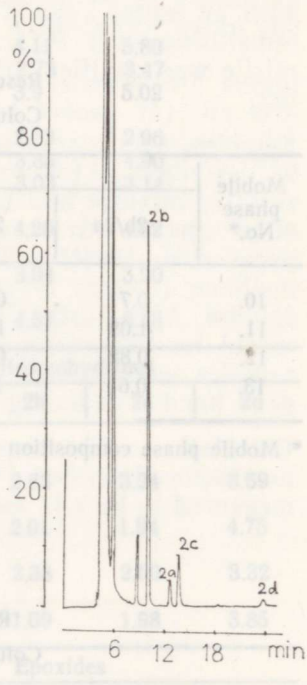

Fig. 3. A chromatogram of the separation of bromohydrines (2). Conditions: column Zorbax SIL (4.6×250 mm); mobile phase: chloroform-isopropanol-water = 93 : 6.93 : 0.07; flow rate 0.8 ml/min; column temperature 35 °C; detection UV 250 nm; absorbance 0.32 AUFS; sample size: the reaction mixture corresponding to 100 μg of the initial dithioketale.

data of mobile phases 10 and 11 showed that the formation of a quaternary system should give complete resolution of all the four compounds. Experimentally it was found that *n*-hexane-ethyl acetate-isopropanol-water (68:9,9:21.1:1.0); mobile phase 12) meets this requirement.

Table 5

Capacity factors (k') of diastereomers formed by the reaction of bromohydrines with Mosher reagent

Column: Zorbax SIL. Mobile phase *n*-hexane-ethyl acetate-isopropanol-water 68:9.9:21.1:1.0. Temperature 35 °C

Diastereomer*	2a	2b	2c	2d
NAT	1.44	0.86	n.s.**	n.s.
ENT	n.s.	1.31	1.73	1.47

* NAT — diastereomer formed with enantiomer leading to natural prostaglandin.
ENT — diastereomer formed with enantiomer leading to ent-prostaglandin.
** n.s. — compound not separated and identified from reaction mixture.

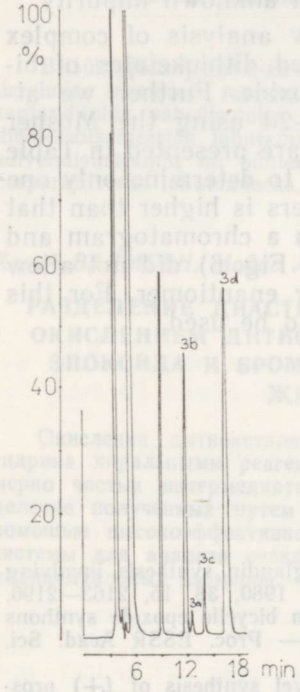

Fig. 4. A chromatogram of the separation of epoxides (3). Conditions: column Zorbax SIL (4.6×250 mm); mobile phase: chloroform-isopropanol-water = 96:3.96:0.04; flow rate 0.8 ml/min; column temperature 35 °C; detection UV 254 nm; absorbance 0.16 AUFS; sample size: the reaction mixture corresponding to 100 μg of the initial dithioketale.

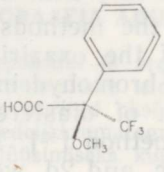

Fig. 5. The chemical structure of the Mosher reagent ((R)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid).

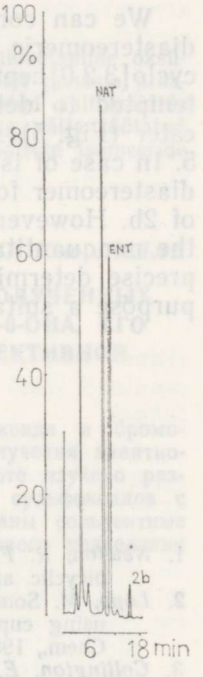

Fig. 6. A chromatogram of the separation of Mosher esters of bromohydrinic sulphoxides 2b. Conditions: column Zorbax SIL (4.6×250 mm); mobile phase: *n*-hexane-ethylacetate-isopropanol-water = 68:9.9:21.1:1.0; flow rate: 0.8 ml/min; column temperature 35 °C; detection UV 260 nm; absorbance 0.16 AUFS; sample size 40 μg. The NAT-diastereomer formed with enantiomer leading to natural prostaglandin. The ENT-diastereomer leading to ent-prostaglandin.

Unfortunately, mobile phase 12 was not applicable to analytical and first step preparative resolution because oxaziridine eluted very near to isomer 2b ($k'=2.34$). For these purposes chloroform-isopropanol-water (mobile phase 13) was suitable (reagent compounds are hardly retentive, see Fig. 3). Using this solvent system the first step of preparative resolution yielded pure isomers 2b and 2d. For complete purification of 2a and 2c, semipreparative chromatography was used (by using mobile phases 10 or 13).

n-Hexane-isopropanol-water was not suitable for the profiling of the formation of diastereomeric epoxides regardless of its most favourable selectivity values (see Table 4; 14). This was caused by the simultaneous retention of oxaziridine and 3d. Therefore, to measure the isomer ratio chloroform-isopropanol-water (mobile phase 15) was used (see Fig. 4). Mobile phases 11 and 15 were both used in the first-step preparative purification (oxaziridine and compounds originating from it are only slightly retained in the column). The fractions collected are reagent-free, but diastereomers 3 could be separated only partly (3b and 3c cannot be resolved by *n*-hexane-ethyl acetate-water). For their final semipreparative separation, *n*-hexane-isopropanol-water mixture (mobile phase 14) suits well. Mobile phase 11 allowed no resolution of isomers 3b and 3c. When mobile phase 15 is used, the purification of a minor isomer, 3d, is disturbed by very near elution of an unknown impurity.

We can conclude that the methods used allow analysis of complex diastereomeric mixtures of the oxaziridine oxidized dithioketales of bicyclo[3.2.0]heptenone, its bromohydrine, and epoxide. Further we attempted to determine e. p. of diastereomers 2a—2d using the Mosher ester (Fig. 5) separation method [12]. The results are presented in Table 5. In case of isomers 2a, 2c, and 2d, we were able to determine only one diastereomer formed. The e. p. of these diastereomers is higher than that of 2b. However, the peaks of the side products on a chromatogram and the nonquantitative course of ester formation (see Fig. 6) did not allow precise determination of the content of the minor enantiomer. For this purpose a suitable chiral separation column should be used.

REFERENCES

1. *Newton, R. F., Roberts, S. M.* Steric control in prostaglandin synthesis involving bicyclic and tricyclic intermediates. — Tetrahedron, 1980, **36**, 15, 2163—2196.
2. *Lopp, M.* Some possibilities of prostanoid synthesis from bicyclic epoxide synthons using cuprate and lithium alkynide·BF_3 reagents. — Proc. ESSR Acad. Sci. Chem., 1987, **36**, 3, 165—171.
3. *Collington, E. W., Wallis, C. J., Waterhouse, I. A.* Novel synthesis of (+) prostaglandin D_2. — Tetrahedron Lett., 1983, **24**, 30, 3125—3128.
4. *Cotterill, I. C., Macfarlane, E. L. A., Roberts, S. M.* Resolution of bicyclo[3.2.0]hept-2-en-6-ols and bicyclo[4.2.0]oct-2-en-endo-7-ol using lipases. — J. Chem. Soc. Perkin Trans. I, 1988, 12, 3387—3389.
5. *Кангер Т. П., Кабат М., Виха Е., Лопп М. И., Лилле Ю. Э.* Оптически активные синтоны для синтеза простаноидов. I. Разделение энантиомеров 2-экзо-бром-3-эндо-гидрокси-бицикло[3.2.0]гептан-6-она. — Ж. орг. хим., 1990, **26**, 8, 1711—1714.
6. *Jäälaid, R., Pehk, T., Kanger, T., Lopp, M., Lille, Ü.* Chiral sulfoxides from dithioketales of bicyclo[3.2.0]hept-2-en-6-one and its epoxide. — Proc. ESSR Acad. Sci. Chem., 1989, **38**, 2, 133—134.
7. *Яалайд Р. Г., Лопп М. И., Пехк Т. К., Лилле Ю. Э.* Оптически активные синтоны для синтеза простаноидов. II. Разделение энантиомеров бицикло-[3.2.0]гепт-ен-6-она через хиральные сульфоксиды. — Ж. орг. хим., 1990, **26**, 12, 2355—2360.

8. *Glahsl, G., Herrmann, R.* (+)-(3-Oxocamphorsulphonyl)oxaziridine as a highly stereoselective reagent for the oxidation of sulphides to chiral sulphoxides. — J. Chem. Soc. Perkin Trans. I, 1988, 6, 1753—1757.
9. *Jäälaid, R., Lopp, M., Pehk, T., Lõhmus, M., Valmsen, K., Lille, Ü.* Asymmetric oxidation of dithioketales of bicyclo[3.2.0]heptanes by (+)-3-(oxocamphorsulphonyl)oxaziridine. In preparation.
10. *Snyder, L. R., Kirkland, J. J.* Introduction to Modern Liquid Chromatography. 2nd ed. New York; Chichester, etc., 1979, 22—43.
11. *Lõhmus, M., Paju, A., Samel, N., Lopp, M., Lille, Ü.* Adsorption high-performance liquid chromatography of prostanoids, use of water-containing mobile phases for separation of 15 R/S-isomers of cloprostenol, and prostaglandins E_1 and E_2. — Proc. ESSR Acad. Sci. Chem., 1986, **35**, 2, 142—148.
12. *David, J. A., Dull, D. L., Mosher, H. S.* α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of alcohols and amines. — J. Org. Chem., 1969, **34**, 9, 2543—2549.

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BITSÜKLO[3.2.0]HEPT-2-EN-6-OONI, SELLE EPOKSIIDI JA BROMOHÜDRIINI DITIOKETAALIDE OKSÜDEERIMISEL MOODUSTUNUD SULFOKSIIDIDE DIASTEREOISOMEERIDE LAHUTAMINE KÕRGSURVEVEDELIKKROMATOGRAAFIA ABIL

Bitsüklo[3.2.0]hept-2-en-6-ooni, selle epoksiidi ja bromohüdriini ditioketaalide oksüdeerimine kiraalsete reagentidega on üks võimalikke teid enantiomeerselt puhaste prostaglandiinide vaheühendite sünteesiks. On uuritud oksüdeerimisel saadud sulfoksiidide diastereoisomeeride lahutamist kõrgsurvevedelikkromatograafia abil ning välja töötatud solvendisüsteemid nii sünteesisegude analüüsimiseks kui ka individuaalsete isomeeride preparatiivseks lahutamiseks.

Карин ВАЛМСЕН, Мадис ЛЫХМУС, Раиса ЯАЛАЙД, Маргус ЛОПП, Юло ЛИЛЛЕ

РАЗДЕЛЕНИЕ ДИАСТЕРЕОИЗОМЕРОВ СУЛЬФОКСИДОВ, ПОЛУЧЕННЫХ ОКИСЛЕНИЕМ ДИТИОКЕТАЛЕЙ БИЦИКЛО[3.2.0]ГЕПТ-2-ЕН-6-ОНА, ЕГО ЭПОКСИДА И БРОМОГИДРИНА МЕТОДОМ ВЫСОКОЭФФЕКТИВНОЙ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ

Окисление дитиокеталей бицикло[3.2.0]гепт-2-ен-6-она, его эпоксида и бромогидрина хиральными реагентами — один из возможных путей получения энантиомерно чистых интермедиатов для синтеза простагландинов. В работе изучено разделение полученных путем асимметрического окисления хиральных сульфоксидов с помощью высокоэффективной жидкостной хроматографии. Разработаны сольвентные системы для анализа реакционных смесей, а также для препаративного разделения индивидуальных изомеров.