Chromatographic separation of the 1-methoxy-2(3)-hydroxy-3(2)-bromocyclohexanes and their derivatives*

During the characterization of the mixture of positionally isomeric and stereoisomeric bromohydrins resulting from the action of aqueous N-bromosuccinimide upon 1-methoxycyclohexene-2[1-3], difficulty was encountered in the separation of the primary products, their acetates, 1-naphthylurethanes, deetherification products, and their diacetates by the conventional methods of distillation and crystallization. Microchemical analysis fails to establish the absence of contamination of a compound by one or more of its isomers and it is frequently difficult to detect small quantities of such undesirable impurities by physical methods such as infrared spectral analysis. In an effort to find a suitable method for the separation of these isomers and to provide reliable criteria for their homogeneity, paper, thin-layer, and vapor phase chromatographic methods were examined and a summary of the results obtained is presented in Table I.

Experimental

(*a*) *Paper chromatography*. The compounds were applied as 200 γ spots (10 λ of an absolute ethanol solution) to 9 in. × 22 in. sheets of Whatman No. 4 paper and were developed by descending flow in an all glass apparatus. With the solvent systems used (see Table I) the chromatograms developed completely in four to six hours. The bromohydrins, bromodiols and diols were detected on the air-dried chromatograms by spraying with 5 % ammoniacal silver nitrate[4] followed by ultraviolet irradiation. This reagent gives better results if the chromatograms are dipped in a 0.1 % solution of iodine in petroleum ether (60–110°) prior to spraying with the silver nitrate solution, since the background then darkens more slowly. The monoacetyl derivatives were detected by spraying the air-dried chromatograms with 2 *N* aqueous potassium hydroxide, followed immediately by light spraying with 0.05 *N* silver nitrate and ultraviolet irradiation. The 1-naphthylurethanes and the bromodiol diacetates were revealed by dipping the chromatograms in the above-mentioned iodine solution.

(*b*) *Thin-layer chromatography*. The compounds were applied as described in (a) to Pyrex glass plates (8 in. × 22 in. × 3/16 in.) coated with a 250 μ layer of silica gel G (Research Specialties Co.) and were developed by ascending flow in an all glass apparatus. The chromogenic agents which had been used to reveal the compounds following paper chromatography proved unsatisfactory for detection on thin-layer chromatograms and had to be modified. The bromohydrins, bromodiols and diols could be detected in quantities as small as 2 γ by spraying air-dried chromatograms with a solution prepared by mixing 4 % aqueous silver nitrate (50 ml), 2 *N* potassium hydroxide (25 ml) and concentrated ammonium hydroxide (15 ml), followed by ultraviolet exposure. Using 500 γ spots during the chromatography of these compounds, it was thus possible to detect as little as 0.5 % contamination by one of its isomers. The bromohydrin monoacetates were revealed by spraying the hot plates (100°) first with ethanolic 2 *N* potassium hydroxide, then with ammoniacal silver nitrate, followed by ultraviolet irradiation. A recognized general method for the detection of

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organic compounds on thin-layer chromatograms is to char them by heating after they have been sprayed with concentrated sulphuric acid[5]. If a 1 % solution of sodium dichromate in 50 % sulphuric acid is used instead of the sulphuric acid, the sensitivity of the method is increased from 5 to 50 tirnes. This procedure was used for detection of the bromohydrin 1-naphthylurethanes and the bromodiol diacetates.

Where N,N-dimethylformamide was used in the solvent system, treatment of the respective support with this chemical prior to chromatography was necessary to ensure reliable results. Papers were dipped in N,N-dimethylformamide–methanol (1:1) and plates for thin-layer chromatography were sprayed with N,N-dimethylformamide–methanol (3:1).

(*c*) *Vapor phase chromatography*. Vapor phase chromatography was performed using an Autoprep A-700 chromatograph coupled with an Electronick 15 Honeywell recorder, employing 3/8 in. aluminum columns which were 5 ft. in length. Samples (5 mg in 50 μl of absolute ethanol) were injected manually and identical conditions (column temperature 150°, collector temperature 150°, injector temperature 168°, detector temperature 175°, filament current 200 mA, helium flow rate 300 ml/min and DMCS-treated Chromosorb P) were used for all the substrates shown in Table I except where otherwise specified. Retention times quoted are those from qualitative experiments. In preparative experiments retention times were somewhat greater depending on the size of sample injected.

Discussion

The results given in Table I demonstrate that it is possible to effect separation of all four isomers in any given group by a judicious choice among the three chromatographic methods examined. It would also appear that vapor phase chromatography (v.p.c.) is more uniformly successful for separation of the isomers of any group in this series than is either of the other methods.

The bromohydrins were not completely separable by paper chromatography with any of the solvent systems examined but separation was readily accomplished by thin-layer chromatography using *n*-hexane–N,N-dimethylformamide–90 % methanol (10:2.5:10). This system was very useful for verification of the homogeneity of the isomers recovered during the preparative v.p.c. separation of bromohydrin mixtures lacking 1α-methoxy-2β-hydroxy-3α-bromocyclohexane on columns employing 30 % XF1150 cyanosilicone as the substrate[3]. In mixtures which contained all four bromohydrins, excellent analytical separations were effected using 10 % Versamid-900 as the substrate and this column proved capable of detecting as little as 0.5 % of any of the isomeric bromohydrins in the presence of the other three after calibration with synthetic mixtures. This column was not, however, suitable for preparative separation of these isomers and for this purpose, 25 % XE60 cyanosilicone was used. Composite substrates containing 15 % Versamid-900 and 5 % XF1150 or 20 % XE60 and 10 % Versamid-900 provided excellent separations initially in preparative experiments using 250 μl of neat bromohydrin mixture per injection, but the resolving power of these columns deteriorated much more rapidly than those in which a single substrate was used.

Some of the substrates proved effective for the v.p.c. analysis of reaction mixtures containing a bromohydrin and its corresponding acetate (30 % XF1150), a bromohydrin and its deetherification product (10 % Versamid-900), a bromodiol and

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TABLE I

Compound	Paper chromatography R_F			Thin-layer chromatography R_F			Vapor phase chromatography retention time (min)			
	Toluene–methyl ethyl ketone–H_2O (1:1:1)	Hexane–95% MeOH (1:1)	Hexane–N,N-dimethylformamide (1:1)	Hexane–N,N-dimethylformamide–95% MeOH (10:2.5:10)	Hexane–95% MeOH (1:1)	Methanol–CCl_4 (1:50)	30% XF1150 Silicone fluid (cyano)	10% Versamid 900	25% DC-11 Silicone grease	25% XE60* Silicone gum (cyano)
trans-2-Bromocyclohexanol				0.09	0.06	0.42	27	8	6	10
1α-Methoxy-2β-hydroxy-3α-bromocyclohexane	0.86	0.47	0.08	0.27	0.01	0.27	93	23	16	30
1α-Methoxy-2α-hydroxy-3β-bromocyclohexane	0.90	0.69	0.15	0.40	0.02	0.37	51	14	13.5	17
1α-Methoxy-2α-bromo-3β-hydroxycyclohexane	0.94	0.40	0.05	0.21	0.03	0.25	92	28	15.5	28
1α-Methoxy-2β-bromo-3α-hydroxycyclohexane	0.94	0.40	0.05	0.36	0.05	0.29	69	17	14	21
1α-Methoxy-2β-acetoxy-3α-bromocyclohexane	Streak	0.73	0.21	0.72	0.04	0.49	180	29	36	59
1α-Methoxy-2α-acetoxy-3β-bromocyclohexane	Streak	0.84	0.49	0.80	0.12	0.67	71	15	26	27
1α-Methoxy-2α-bromo-3β-acetoxycyclohexane	Streak	0.75	0.28	0.75	0.10	0.64	116	22	34	44

1α-Methoxy-2β-bromo-3α-acetoxy-cyclohexane	Streak	0.75	0.28	0.76	0.11	0.60	106	20	30	40
1α-Methoxy-2β-hydroxy-3α-bromocyclo-hexane 1-naphthylurethane	0.96	0.09	0.05	0.05	0.00	0.78				
1α-Methoxy-2α-hydroxy-3β-bromocyclo-hexane 1-naphthylurethane	0.96	0.55	0.30	0.19	0.00	0.51				
1α-Methoxy-2α-bromo-3β-hydroxycyclo-hexane 1-naphthylurethane	0.96	0.35	0.14	0.02	0.00	0.42				
1α-Methoxy-2β-bromo-3α-hydroxycyclo-hexane 1-naphthylurethane	0.96	0.21	0.10	0.02	0.00	0.50				
3α-Bromo-1α,2β-cyclohexanediol	0.64	0.03	0.01	0.04	0.00	0.30		58	13	40
3β-Bromo-1α,2α-cyclohexanediol	0.77	0.00	0.02	0.08	0.02	0.10		44	12	31
2α-Bromo-1α,3β-cyclohexanediol	0.58	0.02	0.01	0.04	0.01	0.05		52	16	42
2β-Bromo-1α,3α-cyclohexanediol	0.46	0.01	0.01	0.01	0.00	0.03		48	15	38
3α-Bromo-1α,2β-diacetoxycyclohexane	0.96	0.86	0.16	0.04	0.08	0.56		39	63	94
3β-Bromo-1α,2α-diacetoxycyclohexane	0.96	0.89	0.26	0.07	0.10	0.58		33	66	82
2α-Bromo-1α,3β-diacetoxycyclohexane	0.96	0.88	0.22	0.09	0.12	0.61		32	61	76
2β-Bromo-1α,3α-diacetoxycyclohexane	0.96	0.85	0.19	0.06	0.09	0.57		39	70	98
cis-1,2-Cyclohexanediol	0.46 (0.62)**	0.08	0.02	0.05	0.00	0.03	40	14	6	10
trans-1,2-Cyclohexanediol	0.33 (0.47)	0.07	0.01	0.03	0.00	0.03	47	17	6	12
cis-1,3-Cyclohexanediol	0.14 (0.23)	0.00	0.00	0.01	0.00	0.00	90	27	8	18
trans-1,3-Cyclohexanediol	0.15 (0.28)	0.00	0.00	0.01	0.00	0.00	80	28	8	19
1β-Methoxy-2α,3α-epoxycyclohexane							9	2	4	3
1α-Methoxy-2α,3α-epoxycyclohexane							19	4	5	6
1,2-Dibromocyclohexane							24	9	12	9
1-Methoxycyclohexene-2							2.5	1	2	1
Cyclohexene							1	0.5	1	5

* On 60/80 mesh Gas-chrom Q (Applied Science Laboratories).

** R_F's in parentheses refer to benzene–methyl ethyl ketone–water (1:1:1).

its diacetate (10 % Versamid-900), or a bromodiol and the corresponding cyclohexanediol (10 % Versamid-900). The best separation of the *cis*- and *trans*-1,2-, and 1,3-cyclohexanediols was obtained when 30 % XF1150 was used. Finally, the complete separation of 1β-methoxy-2α,3α-epoxycyclohexane from 1α-methoxy-2α,3α-epoxycyclohexane, was readily effected using the 30 % XF1150 cyanosilicone substrate. Separation of these isomers by fractional distillation *in vacuo*, on the other hand, always gave appreciable quantities of fractions which contain both of the epoxides.

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1 R. A. B. BANNARD AND L. R. HAWKINS, *Can. J. Chem.*, 36 (1958) 1241.
2 R. A. B. BANNARD AND L. R. HAWKINS, *Can. J. Chem.*, 39 (1961) 1530.
3 R. A. B. BANNARD, A. A. CASSELMAN AND L. R. HAWKINS, *Can. J. Chem.*, 43 (1965) 2398.
4 L. HOUGH, *Nature*, 165 (1950) 400.
5 J. G. KIRCHNER, J. M. MILLER AND G. F. KELLER, *Anal. Chem.*, 23 (1951) 420.

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Book Reviews

Stationary Phase in Paper and Thin-layer Chromatography. Proceedings of the 2nd Symposium held at Liblice, June 10–12, 1964, organised by the Chromatography Group of the Czechoslovak Chemical Society, edited by K. MACEK AND I. M. HAIS, Elsevier, Amsterdam, 1965, 358 pp., price 85 s.

The following five discussions were held at the symposium: chromatography papers, thin-layer materials, stationary liquids and adsorbents in paper chromatography, stationary liquids and impregnations for thin layers, general problems of the stationary phase.

Most papers presented are rather short with the exception of several long accounts mainly by chemists associated with the manufacture of paper or thin-layer materials. The reviewer was very interested in the opinions expressed on such topics as the relative merits of paper versus thin layer separations or the possible applications of glass fibre papers.

On the whole these proceedings are stimulating and interesting although several authors present either summaries or rehashes of previously published work. It might have been useful to show in some way which of the contributions are original and which are not. The volume is a coedition of the Publishing House of the Czechoslovak Academy of Science (who did the actual publishing) and Elsevier. In spite of a lot of editorial work involved such as translations from Russian it appeared rather quickly, less than a year after the symposium.