Chromatographic separation of the 1-alkoxy-2(3)-hydroxy-3(2)-aminocyclohexanes and their derivatives*

In connection with the continuation of our studies on the mode of cleavage of the stereoisomeric 1-alkoxy-2,3-epoxycyclohexanes[1] it became necessary to develop methods for the separation and quantitative estimation of the proportions of the positionally isomeric amines (or suitable derivatives) which could result from the action of ammonia on each oxide. To this end, the paper, thin-layer and vapor phase chromatographic separation of the stereoisomeric 1-alkoxy-2-hydroxy-3-aminocyclohexanes[2], their N-acetyl[2], O,N-diacetyl[2,3] and O,N,N-triacetyl derivatives[3] were examined and the most suitable method was chosen for application to the separation of the position isomers referred to earlier. Results are summarized in Table I.

Experimental

(*a*) *Paper chromatography*

The compounds were applied as 200 γ spots (10 λ of a 2 % solution in absolute methanol) to 9 × 22 in. sheets of Whatman No. 1 paper and developed for approximately 17 h by descending flow in *n*-butanol–acetic acid–water (4:1:5). The chromatograms were dried for 1.75 h at 30° in a forced air oven. Detection of compounds having unsubstituted amino groups was effected by spraying the chromatograms with a 0.04 % solution of bromcresol green (sodium salt) in ethanol. All other compounds were located by dipping the chromatograms in a 0.1 % solution of iodine in petroleum ether.

(*b*) *Thin-layer chromatography*

Each compound, 5 γ (1 λ of a 0.5 % solution in absolute methanol) was applied to 2 × 8 in. strips of Gelman chromatography media Type A (alumina gel on micro glass fibers). The chromatograms were developed (25 min) in hexane–ethyl acetate–methanol (10:1:0.5), unless otherwise specified in Table I, following which they were dried for 2–3 min at 100°. Spraying with a 1 % solution of sodium dichromate in 50 % sulphuric acid and subsequent charring of the chromatograms by heating them on a hot plate produced excellent detection of all the compounds, first as white spots on a yellow-orange background and, on continued heating, as brown spots on a white background.

(*c*) *Vapor phase chromatography*

The instrumentation was the same as mentioned earlier[4] for the vapor phase chromatographic separation of the alkoxybromocyclohexanols but the column packings, temperatures and helium flow rates were different. For column No. 1 Chromosorb P (225 ml, 45–60 mesh) was washed first with concentrated hydrochloric acid (250 ml) for 1 h with occasional stirring, with water (4 × 250 ml), with 10 % sodium hydroxide (250 ml) for 6 h, with water (4 × 250 ml), and finally dried at 130° for 6 h. This material was impregnated with 5 % methanolic potassium hydroxide, dried *in vacuo* at room temperature and again impregnated with 25 % polyethylene glycol 20,000 in chloroform. Column No. 2 was packed with Fluoropak 80 which had been impregnated with 10 % SE 30. The compounds were injected as 1–2 mg samples in 50 μl of absolute methanol.

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Discussion

It is a recorded fact[5] that amines tend to tail badly during vapor phase chromatography on solid supports containing polar sites, presumably due to hydrogen bonding. Unless the Chromosorb P used as the support in some of these experiments was pretreated in a manner similar to that reported by other workers[6,7] it was impossible to elute the amines listed in Table I. Consideration has been given to both the solid support and the stationary phase as the cause of amine tailing[8]. However, it was found that if a common stationary phase (polyethylene glycol 20,000) was used on two different supports, Chromosorb P and Fluoropak 80, complete retention occurred with the former, while elution occurred with the latter. This result indicates that the solid support and not the stationary phase is responsible for the tailing of the amines in the experiments described herein. Column No. 2 was used chiefly for the acetyl derivatives of the amines since even after pretreatment of the solid support Column No. 1 retained virtually all the acetyl compounds.

To determine the relative merits of the three chromatographic methods from the data in Table I, the compounds are considered in groups which consist of stereoisomers and/or position isomers. Paper chromatography with the solvent system used was very effective for the separation of the methoxyaminocyclohexanols (compounds 1, 2 and 3), the ethoxyaminocyclohexanols (11 and 12), the aminocyclohexanediols (19, 20, 21) and somewhat less effective for the aminocyclohexanols (25, 26). However, the acetyl derivatives of all of these compounds moved too rapidly and produced diffuse spots. The latter circumstance made it impossible to distinguish between mixtures of mono- and di-N-acetylamino derivatives (*e.g.* 6 and 9) with this solvent system, but did provide an excellent means of detecting the presence of unreacted amines in their acetylated derivatives.

Thin-layer chromatography was extremely successful for the separation of the acetylated compounds and was especially useful for revealing the contamination of the di-N-acetyl derivatives with their mono-N-acetyl analogues (*e.g.* 6 and 9). By this means it was established that the mono-N-acetyl compounds were present in large amounts in all of the di-N-acetyl derivatives which had been prepared three years previously[3] and which were judged at that time to be free of their mono-N-acetyl analogues on the basis of elemental and acetyl group analysis, and the infrared and n.m.r. spectra of the compounds. It was thus clearly demonstrated that the di-N-acetylamines undergo a slow and spontaneous degradation to their less highly acetylated analogues. The fact that di-N-acetylisopropylamine had undergone complete degradation to mono-N-acetylisopropylamine during this period of storage suggests that some of the lower molecular weight di-N-acetylamines may prove useful as mild reagents for transacetylation. The chromatographic procedures reported herein provide simple analytical methods for studying the kinetics of the decomposition of di-N-acetylamines. Thin-layer chromatography was more effective than either of the other methods examined for the separation of mixtures containing acetylaminocyclohexanols and the corresponding O-acetyl derivatives (*e.g.* 4 and 6). This procedure also provided a better separation for the 1-methoxy-2(3)-acetoxy-3(2)-bromocyclohexanes than the method reported earlier[4]. Although some of the R_F values quoted for the thin layer chromatographic experiments are close, satisfactory separations of the compounds were obtained because of the very compact nature of the spots. It was found that the Gelman support for thin-layer chromatography is very

Compound	Compound No.	Vapor phase chromatography, retention time (min)		R_F	
		Column 1[a]	Column 2[b]	Thin-layer chromatography	Paper chromatography
1α-Methoxy-2β-hydroxy-3α-aminocyclohexane	1	55.5	2.5		0.49
1α-Methoxy-2α-hydroxy-3β-aminocyclohexane	2	48.5	2.5		0.56
1α-Methoxy-2α-amino-3β-hydroxycyclohexane	3	68.0	3.5		0.61
1α-Methoxy-2β-hydroxy-3α-acetylaminocyclohexane	4		15	0.06	0.78
1α-Methoxy-2α-hydroxy-3β-acetylaminocyclohexane	5		14	0.12	0.82
1α-Methoxy-2β-acetoxy-3α-acetylaminocyclohexane	6		16	0.29	0.85
1α-Methoxy-2α-acetoxy-3β-acetylaminocyclohexane	7		18	0.23	0.87
1α-Methoxy-2α-acetylamino-3β-acetoxycyclohexane	8		18	0.70	0.91
1α-Methoxy-2β-acetoxy-3α-diacetylaminocyclohexane	9		31.5	0.61	0.90
1α-Methoxy-2α-acetoxy-3β-diacetylaminocyclohexane	10		21.5	0.90	0.91
1α-Ethoxy-2β-hydroxy-3α-aminocyclohexane	11	54.5	33.5[c]		0.58
1α-Ethoxy-2α-hydroxy-3β-aminocyclohexane	12	44	30.5[c]		0.63
1α-Ethoxy-2β-hydroxy-3α-acetylaminocyclohexane	13		18	0.32	0.83
1α-Ethoxy-2α-hydroxy-3β-acetylaminocyclohexane	14		16.5	0.03	0.86
1α-Ethoxy-2β-acetoxy-3α-acetylaminocyclohexane	15		18	0.59	0.88
1α-Ethoxy-2α-acetoxy-3β-acetylaminocyclohexane	16		18.5	0.49	0.89
1α-Ethoxy-2β-acetoxy-3α-diacetylaminocyclohexane	17		34.5	0.74	0.90
1α-Ethoxy-2α-acetoxy-3β-diacetylaminocyclohexane	18		24	0.93	0.91
3α-Amino-1α,2β-cyclohexanediol	19		3.5		0.36
3β-Amino-1α,2α-cyclohexanediol	20		2.5		0.40
2α-Amino-1α,3β-cyclohexanediol	21		4.5		0.45
3α-Acetylamino-1α,2β-diacetoxycyclohexane	22		26.5	0.60[d]	0.87
3β-Acetylamino-1α,2α-diacetoxycyclohexane	23		25.0	0.67[d]	0.88
2α-Acetylamino-1α,3β-diacetoxycyclohexane	24		30	0.48	0.89
trans-2-Aminocyclohexanol	25	30	10[c]		0.58
cis-2-Aminocyclohexanol	26	27.5	10.5[c]		0.61
trans-2-Acetylaminocyclohexanol	27		8.0	0.19	0.83
cis-2-Acetylaminocyclohexanol	28		7.5	0.26	0.85
1α-Acetoxy-2β-acetylaminocyclohexane	29		9.0	0.53	0.89
1α-Acetoxy-2α-acetylaminocyclohexane	30		11.5	0.37	0.89
1α-Acetoxy-2β-diacetylaminocyclohexane	31		17.0	0.44	0.93
trans-2-N-Ethylacetylaminocyclohexanol	32	14.5		0.47	0.90
1α-N-Ethylacetylamino-2β-acetoxycyclohexane	33	21.5	70	0.73	0.92
Cyclohexylamine	34	3.5	0.5		0.72
Acetylaminocyclohexane	35		3.5	0.68	0.91
Diacetylaminocyclohexane	36		6.5	0.94	0.92
Ethylacetamide	37	17	3.5[c]	0.45	0.85
Ethyldiacetamide	38	17	8.0[c]	0.89	0.89
Isopropylacetamide	39	14.5	4.0	0.52	0.87
Benzyldiacetamide	40		2.5, 12.0	0.54, 0.88	0.91

[a] Conditions: column 165°; collector 158°; detector 185°; injector 180°; He flow 300 ml/min. [b] Conditions: column 170°; collector 160°; detector

satisfactory since it can be treated with sulphuric acid and exposed to high charring temperatures without the danger of breakage experienced by the use of glass plates.

Vapor phase chromatography on column 1 provided an excellent method for separation of the alkoxyaminocyclohexanols and aminocyclohexanols. Most noteworthy in this connection is the capability of the procedure to separate the three methoxyaminocyclohexanols on a preparative scale, thereby providing a suitable means for the isolation of the difficultly accessible 1 α-methoxy-2 α-amino-3 β-hydroxycyclohexane[1] in a pure condition. This column was not satisfactory for separation of the acetylated derivatives but the latter compounds were resolved when column 2 was used. The relatively short retention times achieved by the use of this column made for rapid analysis and the method was convenient for checking the products from the degradation of the di-N-acetylamino compounds. This column was not satisfactory, however, for the separation of the alkoxyacetylaminocyclohexanols from their O-acetyl derivatives (*e.g.* 13 and 15) but these compounds are readily separable by the thin-layer chromatographic method mentioned earlier.

It is evident from Table I that by selecting one of the three chromatographic methods examined, any individual member of any of these groups of isomers can be separated. These results underline the importance of concurrent examination of several chromatographic techniques when difficulty is encountered in the separation of isomers by more conventional means.

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Stabilization of xanthophyll and carotene by ethoxyquin during thin-layer chromatography

The use of thin-layer adsorption chromatography (TLC) for the quantitative and qualitative determination of xanthophyll and carotene is limited due to the rapid oxidation and isomerization of these compounds during TLC. Employment of impregnated and reverse-phase TLC[1] as well as saturated solvent chambers[2] has eliminated some of these losses, however, these procedures are more complex and require more time and equipment than the more common technique of adsorption chromatography. Recently, a procedure was described for retarding autoxidation of lipids