

Chromatographic separation of hydrogenation products of dibenz[*a,h*]-anthracene

Evidence shows that some partially hydrogenated derivatives of dibenz[*a,h*]-anthracene are effective in decreasing tumor incidence when administered with the highly carcinogenic parent hydrocarbon^{1,2}. As a part of a program to prepare half-gram amounts of several of these derivatives by catalytic hydrogenation of dibenz[*a,h*]anthracene, methods for separating the hydrogenation products from the parent compound and each other were required. This report describes our efforts to develop gas and column chromatographic methods for carrying out the large-scale separation.

Development column chromatographic techniques were used by LIJINSKY³⁻⁶ for separating reduced derivatives of dibenz[*a,h*]anthracene. The basis of his separation was development on magnesia-Celite, after which the column material was extruded, and the segments containing the various derivatives were separated, eluted, and rechromatographed. In order to separate larger amounts of materials more rapidly, two continuous separation techniques, preparative gas chromatography and elution column chromatography, were selected for evaluation in preference to the successful but slower technique of LIJINSKY.

Several unsuccessful attempts were made to separate these hydrogenation products by preparative gas chromatography. All of the experiments were conducted on a Wilkens Model 700 Autoprep preparative gas chromatograph fitted with a 3/8 in. × 30 in. column packed with 10% SE-30 (a G.E. methyl silicone) on 60-80 mesh acid-washed Chromosorb W (Applied Science Laboratories, State College, Pa.).

Three problems combined to make this approach unsuccessful. First, the reaction products and the parent compound are solids at room temperature, and only slightly soluble in organic solvents. Thus they had to be injected as dilute solutions (0.5 ml of benzene) so that yields on each cycle were low, and much solvent in the exit stream had to be trapped and removed.

Secondly, the high melting points of the parent compound and the derivatives (e.g., dibenz[*a,h*]anthracene, 262°; 1,2,3,4-tetrahydrodibenz[*a,h*]anthracene¹, 211-212.5°) required extremely high injector, column, detector, and collector temperatures and short columns in order to obtain symmetrical peaks, short retention times (to permit repetitive injections), and adequate resolution. The best separations were achieved at 345°, at which temperature the stationary phase bled too rapidly; the nonmetallic parts of the instrument, especially the gaskets, deteriorated rapidly; and evidence from repetitive injections of trapped fractions indicated that the hydrogenation products had partially decomposed.

Thirdly, the parent compound and hydrogenation products apparently existed in the exit stream as aerosols and were extremely difficult to trap in any standard collector. Many modifications of the collectors were made but much potentially carcinogenic material always penetrated the collection system so that the latter had to be installed in a special hood.

Both silica⁷⁻¹¹ and alumina^{9, 10-21} have been used for separating benzo[*a*]pyrene and dibenz[*a,h*]anthracene from other polynuclear aromatic hydrocarbons by liquid adsorption column chromatography. Alumina produced the best separations with mobile phases consisting of mixtures of aromatic and alicyclic or aliphatic hydro-

carbons, or petroleum ether and acetone, benzene-cyclohexane being the most widely used combination.

Because of the previous successes with alumina, and of the expectation that the separation of these structurally similar partially hydrogenated derivatives of dibenz[*a,h*]anthracene from each other and the parent compound would be more difficult than the separation of polynuclear aromatic hydrocarbons, acid-washed alumina (Merck) was selected for this separation. To ensure a standard adsorbent, all of the acid-washed alumina was heated at 130° for 30 h, after which 1.7 % water was added to give a total of 13.5 %²². A short experiment on 7 mm × 40 mm columns showed that dibenz[*a,h*]anthracene could be eluted from this alumina with no apparent decomposition.

A mixture of benzene-cyclohexane was similarly selected as the eluent. Experiments on the 7 mm × 40 mm columns with a hydrogenated dibenz[*a,h*]anthracene mixture showed that a mixture of 15 % benzene and 85 % cyclohexane (by volume) gave adequate separation as judged by development of fluorescent bands on the column, consistent with a desirable slow flow rate.

Other experimental conditions selected for separation were: column size, 1 in. × 36 in.; column packing, 475 g of standardized alumina in a slurry of eluent (benzene-cyclohexane, 15:85); column loading, 5 g of hydrogenated dibenz[*a,h*]anthracene mixture in a slurry with 300 ml of the eluent (although this technique is not ideal, it was permissible because the most soluble solutes were the least strongly adsorbed, and the least soluble—dibenz[*a,h*]anthracene—was the most strongly adsorbed); column flow rate, not controlled but varied between 2 and 60 ml/h. In addition, the eluent was kept saturated with nitrogen to reduce oxidation on the column, and the column was covered with aluminum foil to eliminate light and consequent decomposition.

Fractions were collected with Packard and LKB automatic constant-volume fraction collectors. In some experiments, unresolved mixtures were rechromatographed on the same type of column with the same eluent. All the fractions containing single components or similar mixtures were combined and the eluent was removed with a flash evaporator. The various hydrogenation products and the parent compound were identified in the fractions by NMR spectroscopy and ultraviolet spectroscopy, using for comparison ultraviolet spectra of pure compounds furnished us by Dr. LIJINSKY.

Elution order was established by chromatography and rechromatography of several hydrogenated dibenz[*a,h*]anthracene mixtures, *i.e.* a mixture of highly hydrogenated unidentified materials, 5,6,12,13-tetrahydrodibenz[*a,h*]anthracene, 1,2,3,4,12,13-hexahydrodibenz[*a,h*]anthracene, 5,6-dihydrodibenz[*a,h*]anthracene, 1,2,3,4-tetrahydrodibenz[*a,h*]anthracene, and finally dibenz[*a,h*]anthracene. Not all of these compounds could be separated by repetitive column chromatography, and separations were terminated when the following pure compounds and mixtures were obtainable:

- (a) 5,6-dihydrodibenz[*a,h*]anthracene, 99+ mol %;
- (b) 5,6-dihydrodibenz[*a,h*]anthracene, 86.5 ± 2.5 mol %, and 1,2,3,4-tetrahydrodibenz[*a,h*]anthracene, 13.5 ± 2.5 mol %;
- (c) 1,2,3,4,12,13-hexahydrodibenz[*a,h*]anthracene (72.5 ± 5 mol %), and 5,6,12,13-tetrahydrodibenz[*a,h*]anthracene (27.5 ± 5 mol %).

Estimates of the purity of these compounds were obtained by integration of NMR spectra.

Conclusion

The column chromatographic method is simpler than previously published methods for separating partially hydrogenated derivatives of dibenz[*a,h*]anthracene, and it gives satisfactory separations. Several of the compounds were not completely separated by the method; operating the column at optimum conditions for separation²³ or use of solvent gradients, should improve the separation. Neither of these approaches was tried in this study.

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