matographic separation of the 14 and 16 carbon 1,2-diol standards synthesized by the method of SWERN, BILLEN AND SCANLON[3]. (A) represents a scan obtained using an 1/8 in. × 6 ft. copper column packed with 7 % Zonyl E-7 coated on Chromosorb W H.P., 80–100 mesh (Varian-Aerograph, Walnut Creek, Calif.). Scan (B) was from a copper column, 1/8 in. × 3 ft. packed with 2 % Versamid 900 on Chromosorb AW-DMCS, 80–100 mesh (Hewlett-Packard-F&M, Avondale, Pa.). Chromatogram (C) was from an 1/8 in. × 3 ft. copper column packed with 2 % OV-17 on Anakrom Q, 60–70 mesh (Analabs, Hamden, Conn.). Analyses were obtained using a HyFi 600 gas chromatograph (Varian-Aerograph, Walnut Creek, Calif.) equipped with a hydrogen-flame ionization detector with helium as the carrier gas at a flow rate of 30 ml/min in all cases. Column temperature conditions were: (A) 195°; (B) 195°; (C) 160°.

Use of these columns allowed us to identify 1,2-diols of 14 through 18 carbon atoms obtained from the biological oxidation of 1-alkenes of 14 through 18 carbons by *C. lipolytica*.

Financial support was from N.I.H. grant AI-06707.

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Received May 24th, 1968

J. Chromatog., 36 (1968) 341–342

CHROM. 3583

The identification of glycerol, ribitol and anhydroribitol by gas–liquid and thin-layer chromatography

The difficulty of identifying the ribitol and glycerol components of cell wall hydrolysates has been outlined by IKAWA, MORROW AND HARVEY[1]. We have now found that the application of accepted gas chromatographic methods, using trimethylsilyl derivatives[2], to this problem yields excellent separations, as also does the use of the same derivatives in thin–layer chromatography. Similar TLC techniques in the analysis of other sugars have been reported by LEHRFELD[3].

Methods and results

A mixture of ribitol and anhydroribitol was prepared by hydrolysing 5 mg of ribitol (Lights) with 2 ml of 2 *N* HCl in a sealed tube at 110° for 3 h. The products were dried by rotary evaporation followed by the addition and evaporation of chloroform (B.D.H. redistilled and stabilised by the addition of 1 % absolute ethyl alcohol).

Trimethylsilyl derivatives of the dried material and of 5 mg each of ribitol and glycerol (B.D.H., AnalaR) were prepared by dissolving each sample in 0.2 ml of pyridine (B.D.H. AnalaR) and adding successively 0.1 ml hexamethyl disilazane (Hopkins and Williams) and 0.05 ml trimethylsilyl chloride (Hopkin and Williams). After shaking for a few minutes each sample was centrifuged to compact most of the precipitate and the pyridine was removed from the supernatants in a rotary evaporator at 45°. After redissolving in chloroform and reevaporating, the products were dissolved in 0.2 ml each of chloroform.

Gas chromatography of 1 μl aliquots on a 5 ft., 3.5 % SE 52 on 85–100 mesh silanised Diatomate-C, glass column at 130°, with a carrier gas flow rate of 45 ml/min gave the following retention times:

Ribitol 32.8 min and 38.5 min (two peaks).
Anhydroribitol 11.7 min.
Glycerol 2.7 min.

Thin-layer chromatography of 2 μl aliquots, in benzene, on 6.7 cm × 2.5 cm or 10 cm × 5 cm pieces of Merck Silica Gel F_{254} precoated plates, gave spots which were visualized by U.V. fluorescence as the plates dried, and by 50 % aqueous sulphuric acid followed by charring at 150°. R_F values were approximately:

Ribitol 0.92.
Anhydroribitol 0.34.
Glycerol 0.67.

Anhydroribitol and glycerol were easily resolved in spite of some streaking. The identity of each spot was confirmed by GLC of a benzene extract from the plate.

Conclusion

A rapid distinction can be made between glycerol and anhydroribitol by thin-layer chromatography of the trimethylsilyl ethers. Quantitative measurement employing similar derivatives is now standard practice in the GLC of other compounds and, with the use of suitable calibrations and, perhaps, internal standards, should present no special difficulties with anhydroribitol and glycerol.

Acknowledgements

I am grateful to Drs. C. HUGHES and J. GARRETT for stimulating my interest in this work and to P. RECKLESS and KATHLEEN YOUNG for technical assistance.

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Received April 24th, 1968