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Note

Use of bromine as an aid in the identification of unsaturated fatty alcohols and diols

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Bromination of fatty acids, alcohols and diols is a rapid, simple and quantitative procedure. Brominated fatty acids of different double bond composition have been analysed by thin-layer chromatography (TLC)[1]. Gas chromatographic analysis of brominated long-chain fatty compounds is generally restricted to molecules with one or two double bonds[2], due to the polarity and high molecular weight of the addition products. Changes in retention seen after bromination, however, can be a valuable aid to establishing the identity and double bond composition of such compounds. The bromination technique is applied here to the analysis of diols obtained by reducing linoleic acid hydroperoxide with lithium aluminium hydride.

MATERIALS AND METHODS

Fatty acids and their 12-hydroxy derivatives were obtained from Sigma (St. Louis, Mo., U.S.A.). Linoleic acid hydroperoxide was prepared by air oxidation of the free fatty acid. After separation of the hydroperoxide from parent acid using TLC on silica plates (Merck, Darmstadt, G.F.R.) with isooctane–ethyl acetate (1:1) as mobile phase, the hydroperoxide was reduced using an excess of lithium aluminium hydride in dry diethyl ether. Excess reagent was destroyed using 0.1 *M* HCl and the products extracted into diethyl ether and taken to dryness under a stream of nitrogen. Both the hydroperoxide and corresponding conjugated diol gave a characteristic absorption maximum at 234 nm. Fatty acids and hydroxy fatty acids were reduced to the corresponding alcohols using the same procedure. Extracts were brominated using carbon tetrachloride–bromine (50:1) for 5 min at room temperature. Excess bromine and solvent were removed under a stream of nitrogen and the bromo compounds derivatised using heptafluorobutyric anhydride (Pierce, Rockford, Ill., U.S.A.) in dry ether at 60° for 10 min.

Chromatography was performed using a 7-m glass capillary column coated with SE-52 in a Carlo Erba chromatograph maintained at 215°. Detection was by electron capture using nitrogen as carrier.

RESULTS AND DISCUSSION

Fig. 1 shows the retention of brominated alcohols of varying carbon number and degree of unsaturation. Bromo-derivatives of *cis* and *trans* monoenes of a given carbon number are not resolved under these conditions[2]. Addition products obtained with diene alcohols, however, have 4.22-fold greater retention than the corresponding monoene compound, whilst the introduction of a hydroxyl group into a monoene alcohol derivative actually decreases the retention time by 8% despite the increase in molecular weight.

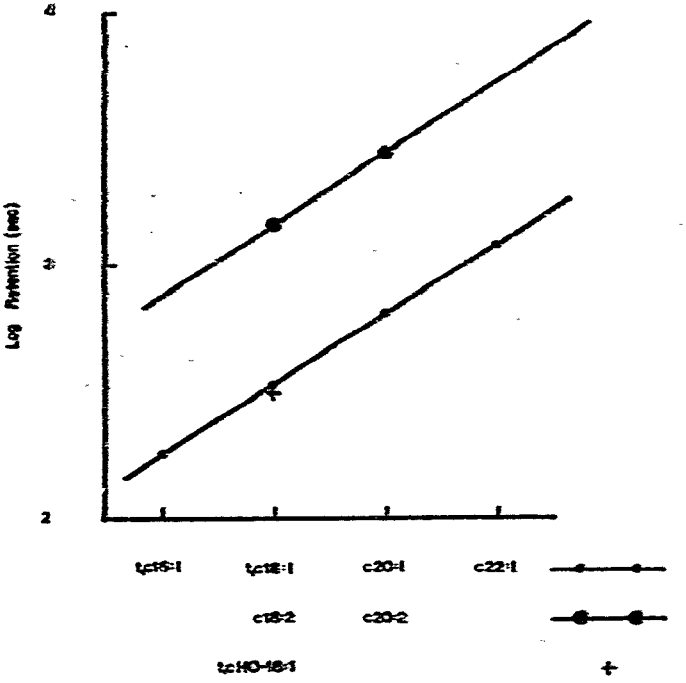

Fig. 1. Retention times of brominated fatty alcohols and diols. Fatty alcohols and diols were brominated and then derivatised with heptafluorobutyric anhydride. These derivatives were analysed using a 7-m glass capillary column coated with SE-52 mounted in a Carlo Erba gas chromatograph maintained at 215°. Detection by electron capture. t = *trans*, c = *cis*.

Air oxidation of linoleic acid yields a mixture of hydroperoxides with a conjugated double bond system[3]. Under the conditions used in these experiments, the bromination products with diols obtained by reducing linoleic acid hydroperoxide have the same retention time as brominated 9-octadecaene-1,12-diol, indicating that only one double bond equivalent has undergone addition with halogen.

Fig. 2 shows the application of this bromination technique to a mixture of diols derived from a tissue culture cell extract of cells grown in serum-free medium supplemented with linoleic acid bound to bovine serum albumin[4]. Peaks A and C are unaffected by bromination and correspond to 16- and 18-carbon fatty acids hydroxylated in the w and w-1 positions[5]. Peak B is absent when linoleic acid is excluded from the culture medium. Peak D represents the bromination product of compound B and has the same retention as a brominated 9-octadecaene-1,12-diol, suggesting

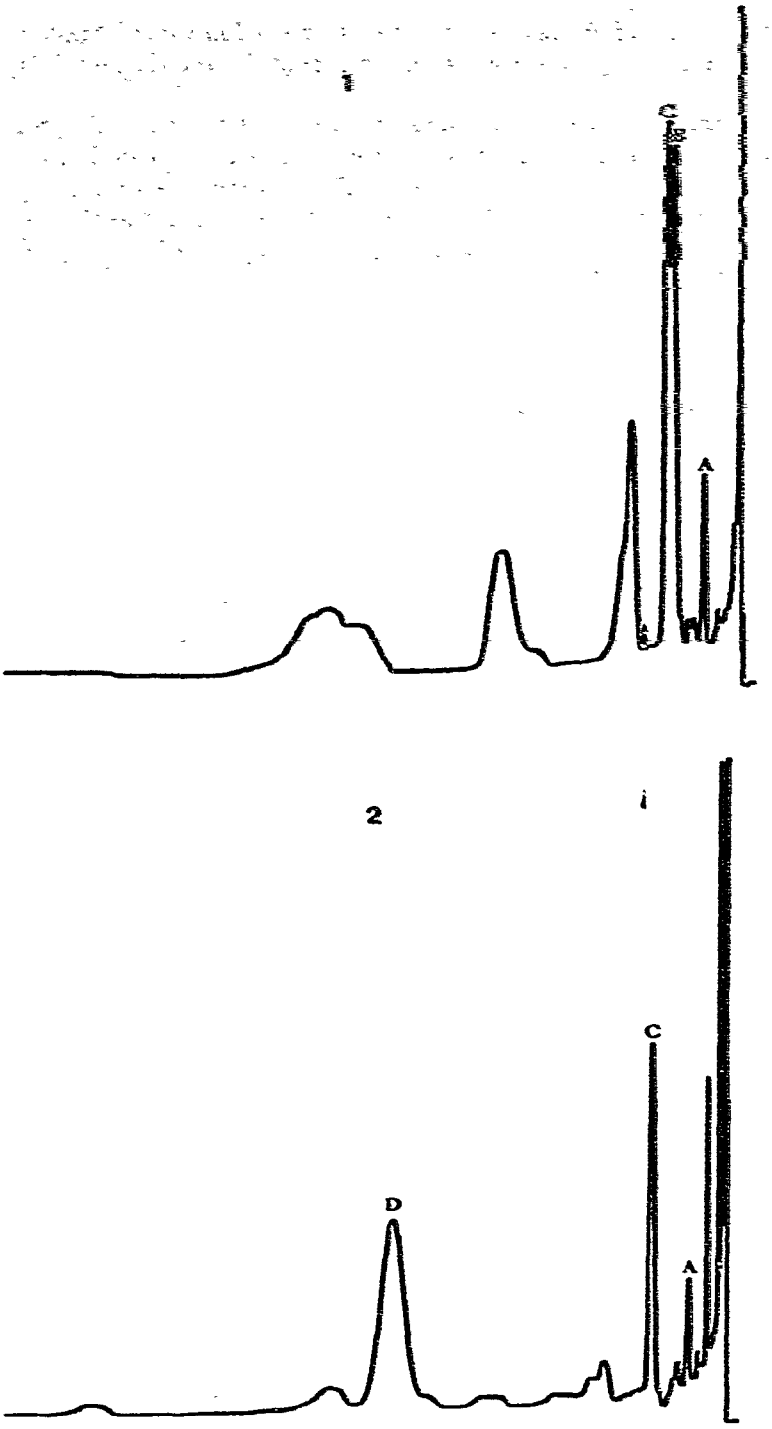

Fig. 2. Diols obtained from tissue culture cells supplemented with linoleic acid. (1) Before bromination, (2) after bromination. Oven temperature 200°.

that compound B is a conjugated diene formed during the peroxidation of linoleic acid rather than by the direct hydroxylation of linoleic acid in the w and w-1 positions.

In conclusion, bromination will only distinguish between fatty alcohols and diols containing one or two double bonds if these double bonds are isolated (*e.g.*, methylene interrupted). Conjugated dienes only react with bromine as though one double bond is present, possibly as a result of halogen exchange during addition to the second double bond. Consequently, conjugated and non-conjugated long-chain fatty dienes may be distinguished by this method.

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REFERENCES

1 L. Gosselin and J. De Graeve, *J. Chromatogr.*, 110 (1975) 117.
2 R. A. Stein, *J. Chromatogr.*, 6 (1961) 118.
3 H. W. S. Chan and G. Levett, *Lipids*, 12 (1977) 99.
4 R. J. Chenery and A. E. M. McLean, *Biochim. Biophys. Acta*, 572 (1979) 9.
5 R. A. Prough, R. T. Okita, L. L. Fan and B. S. S. Masters, *Methods Enzymol.*, 52C, p. 318.