

Direct Methylation of Salts of Acids in Hydrolysis Mixtures of Natural Products by Phase Transfer Catalysis. An Investigation of the Suberin Fraction of Birch Bark (*Betula verrucosa* Erh.)

INGMARI BOLMGREN and
TORBJÖRN NORIN

Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden

Epoxy acids have been detected in several cutins and suberins. No direct method to determine the amounts of epoxy acids present has been reported. Holloway¹ and Kolattukudy,^{2,3} however, estimated the amounts of these acids in different cutins and suberins *via* indirect methods. In this communication we report the use of phase-transfer catalyst for the direct preparation of methyl esters of epoxy acids in the hydrolysis mixture from suberin of birch (*Betula verrucosa* Erh.). A quantitative estimation of the epoxy acids and other constituents from the suberin can then be made using TLC and GLC analysis.

The suberin fraction from bark of *Betula verrucosa* Erh. was hydrolysed in the usual way by ethanolic potassium hydroxide (3%). A slight excess of tetrabutylammonium hydroxide was added to one half of the filtered hydrolysis mixture. The solution was evaporated and the residue was extracted with dichloromethane containing a slight excess of methyl iodide.⁴ After evaporation of the solvent the methyl esters were extracted with diethyl ether. A TLC analysis (silica gel; ethyl acetate–chloroform, 3:7) of the methyl esters showed the presence of diacids ($R_f = 0.65 - 0.85$), hydroxy acids ($R_f = 0.42 - 0.50$) and 9,10-epoxy-18-hydroxyoctadecanoic acid ($R_f = 0.35 - 0.40$).

The other half of the hydrolysis mixture was acidified, extracted with diethyl ether and methylated with diazomethane in the usual manner. Only a very small amount of epoxy acid could be detected. A spot ($R_f = 0.24 - 0.29$) corresponding to the hydrolysis products of 9,10-epoxy-18-hydroxyoctadecanoic acid was now present. The experiment shows that the formation of diols essentially occurs on acidification.

It has been suggested that the aqueous hydrolysis of suberin will open the epoxide rings to yield diols.^{5,6} We have performed an analysis of suberin from birch bark using aqueous alkali for the

hydrolysis and the ion-pair method to methylate the acids. A TLC analysis showed the presence of epoxy acids, although the amounts were slightly less than in the experiment using alcoholic alkali for the hydrolysis. Thus the analysis of sensitive epoxy acids in cutins, suberins and similar natural lipid products can readily be performed after hydrolysis with alcoholic alkali, followed by methylation of the acid salts in the hydrolysis mixture using a phase transfer catalyst.

1. Holloway, P. J. and Brown Deas, A. M. *Phytochemistry* 12 (1973) 1721.
2. Kolattukudy, P. E., Walton, T. J. and Kushwahs, R. P. S. *Biochem. Biophys. Res. Commun.* 42 (1971) 739.
3. Walton, T. J. and Kolattukudy, P. E. *Biochemistry* 11 (1972) 1885.
4. Brändström, A. *Preparative Ion Pair Extraction*, Swedish Academy of Pharmaceutical Sciences, Stockholm 1974.
5. Guillemonat, A. and Cesaire, G. *Bull. Soc. Chim. Fr.* 16 (1949) 792.
6. Seoane, E. and Ribas, I. *An. R. Soc. Esp. Fis. Quim. Ser. B* 47 (1951) 61.

Received October 1, 1981.