conditions and thus by measuring the rate of change of the colour of an unknown sample to estimate its age by extrapolation. Not enough results are, however, available from the present investigation to enable this to be done.

I wish to thank Mr. S. S. Kind, of the Home Office Forensic Science Laboratory, Harrogate, for helpful discussions.

D. PATTERSON
Department of Colour Chemistry and Dyeing,
University of Leeds,
Leeds, 2.

[1] Polson, C. J., "Essentials of Forensic Medicine", 206 (English University Press, 1955).
[2] Taylor, A. S., "Principles and Practice of Medical Jurisprudence", 1, 357 (1956).
[3] Glaister, J., "Medical Jurisprudence and Toxicology", 304 (1957).
[4] Glasser, L. G., and Troy, D. J., *J. Opt. Soc. Amer.*, **42**, 652 (1952).

Determination of Fatty Acids of Lower Molecular Weight by Gas Chromatography

ANALYTICAL methods for the qualitative and quantitative analysis of fatty acids have been concerned primarily with the fatty acids of higher molecular weight because of their importance in health and nutrition. Small quantities of fatty acids as the methyl esters can be separated by the gas-liquid partition chromatography of James and Martin[1,2]. The fractionation is based on the length of the carbon chain with the more recent development, including fractionation on the basis of unsaturation[3–5]. These methods, however, do not provide for the convenient analysis of the acids of lower molecular weight in a mixture of fatty acids. By modification of the method of Craig and Murty[5] it is possible to separate and identify the fatty acids of lower molecular weight with the same column as that used for the separation of the acids of higher molecular weight.

An 'Aerograph' gas chromatographic instrument, model *A*-100, was employed for the fractionation of the methyl esters of the fatty acids. The stainless steel column, ¼ in. outer diameter and 10 ft. long, was packed with 'Chromasorb', 60–80 mesh (Johns-Manville, Celite Division, New York), impregnated with polyester butanediol succinate[5]. 5 gm. of the polyester dissolved in 200 ml. of acetone were added to 25 gm. of 'Chromasorb'. The acetone was removed by heating on a steam-bath, after which the mixture was dried in an oven at 110° C. for 6 hr. A filament current of 210 m.amp. was maintained on the standard 'Aerograph' four-filament detector. A 'Varian' 10 mV. recorder was modified with a range selector switch to give a 1 mV. full-scale sensitivity.

The original procedure was modified for the separation of the lower molecular weight esters. A known mixture of fatty acids, consisting of acetic, propionic, *iso*butyric, butyric, 2-methyl butyric, *iso*valeric, valeric, *iso*caproic, caproic, enanthic, and caprylic acids, were converted to the corresponding methyl esters by reaction with diazomethane, using the microtechnique of Roper and Ma[6]. Since these esters would be characterized by lower boiling points, the column was maintained at a temperature of 92° C. with helium used as the carrier gas at a flow-rate of 40 ml. per min.

A chromatogram showing the separation of these lower molecular weight fatty acid esters is shown in Fig. 1. Using this technique, it is possible to separate the lower molecular weight fatty acids in approximately 28 min.

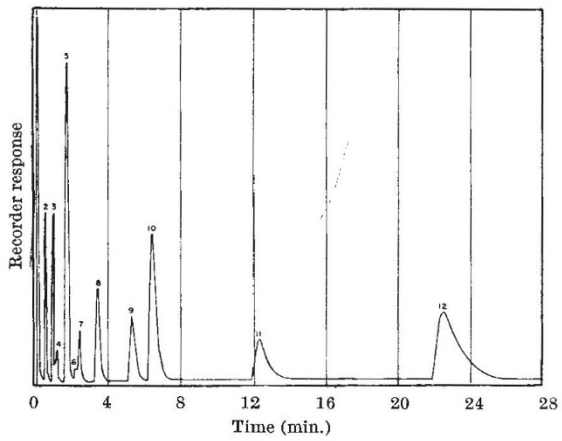

Fig. 1. Separation of lower molecular weight fatty acid esters. 1, Solvent (diethyl ether); 2, methyl acetate; 3, methyl propionate; 4, methyl *iso*butyrate; 5, methyl butyrate; 6, methyl-2-methylbutyrate; 7, methyl *iso*valerate; 8, methyl valerate; 9, methyl *iso*caproate; 10, methyl caproate; 11, methyl enanthate; 12, methyl caprylate. Efficiency: 3,320 theoretical plates (methyl caprylate)

The separation of the higher molecular weight fatty acids can be accomplished by raising the temperature to 216° C. and increasing the flow-rate to 60 ml. per min. The column is brought to equilibrium and another sample injected into the column.

By comparing the peak area of an ester such as methyl caprylate which appears on both chromatograms it is possible to establish a quantitative relationship between the lower and higher molecular weight fatty acids. If no peak overlaps, the addition of a known amount of methyl caprylate will establish a reference point.

MARIE L. VORBECK
LEONARD R. MATTICK
FRANK A. LEE
CARL S. PEDERSON

New York State Agricultural Experiment Station,
Cornell University,
Geneva,
New York.

[1] James, A. T., and Martin, A. J. P., *Biochem. J.*, **50**, 679 (1952).
[2] James, A. T., and Martin, A. J. P., *Analyst*, **77**, 915 (1952).
[3] Orr, C. H., and Callen, J. E., *J. Amer. Chem. Soc.*, **80**, 249 (1958).
[4] Lipsky, S. R., *Chem. Eng. News*, **36** (No. 5), 48 (1958).
[5] Craig, B. M., and Murty, N. L., *J. Amer. Oil Chem. Soc.*, **36**, 549 (1959).
[6] Roper, R., and Ma, T. S., *Microchem. J.*, **1**, 581 (1957).

BIOCHEMISTRY

Microbiological Transformation of Terpenes: Hydroxylation of α-Pinene

THE microbiological transformations of steroids and alkaloids have opened up a new line of approach towards the synthesis of many biologically active compounds. Relatively little is known, however, about the capability of micro-organisms to transform the simple terpenoid compounds. Recently, Bradshaw *et al.*[1,2] have studied the microbiological transformation of camphor.

The yields of essential oils from some oil-bearing plants are known to be considerably enhanced if the plants are infected by micro-organisms[3]. The present communication deals with the microbiological transformations of α-pinene by moulds.