# Extractives from East Africa Timbers. Part III.[1] The Identification of Volatile Acids from Complex Natural Esters

By D. H. Calam and D. A. H. Taylor

Hydrolysis of the complex natural product bussein has given acetic, isobutyric, and α-methylbutyric acids, and a non-volatile residue. A simple n.m.r. spectrometric method for analysis of the volatile acids is described.

MANY natural products, such as the veratrum alkaloids,[2] consist of esters of polyhydric alcohols with several different simple acids. A compound of this type is bussein,[3] now shown by the mass spectrum [4] to have the formula $C_{44}H_{58}O_{18}$. Mild alkaline hydrolysis of bussein gave a mixture of aliphatic acids, identified by conversion into their *p*-phenylphenacyl esters which were separated by chromatography in a way similar to that used for the veratrum alkaloids.[5] The esters of acetic, isobutyric, and D-(—)-α-methylbutyric acids were obtained crystalline in the ratio 3·36 : 1·0 : 1·36. Difficulty was experienced in the separation of the esters of the last two acids, which formed sharply melting mixed crystals. The n.m.r. spectra of such mixed crystals showed their nature clearly, and provided a convenient method of analysing them. This suggested that the whole analysis could be performed by n.m.r. examination of the mixed acids.

A sample of bussein was hydrolysed by boiling with a known excess of alkali in aqueous methanol, and the solution was then treated with the equivalent amount of dilute sulphuric acid. The neutralised solution was then evaporated to dryness under reduced pressure, and titration showed that the distillate contained 5 molecular equivalents of acid. When the hydrolysis solution was steam-distilled, but not evaporated completely to dryness, less volatile acid was obtained. Evaporation of the titrated, neutral distillate under reduced pressure

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[1] Part II, F. M. Dean and D. A. H. Taylor, *J. Chem. Soc.* (*C*), 1966, 114.

[2] L. F. Fieser and M. Fieser, "Steriods," Reinhold, New York, 1959, p. 880.

[3] D. A. H. Taylor, *J. Chem. Soc.*, 1965, 3495.

[4] F. M. Dean, personal communication.

[5] G. S. Myers, W. L. Glen, P. Morozovitch, R. Barber, G. Papineau-Couture, and G. A. Grant, *J. Amer. Chem. Soc.*, 1956, **78**, 1621.

gave a solid residue of sodium salts, which was examined in deuterium oxide solution in a Varian A56/60 n.m.r. spectrometer. The spectrum showed bands at $\delta$ 2·3 (quintuplet; $CO{\cdot}C\mathit{H}R_2$; isobutyrate and α-methylbutyrate), 2·0 (singlet; acetate), 1·4 (multiplet; $CH_3{\cdot}C\mathit{H}_2{\cdot}CHR_2$; α-methylbutyrate), 1·1 (doublet; $C\mathit{H}_3{\cdot}CHR_2$; isobutyrate and α-methylbutyrate), and at 0·9 p.p.m. (multiplet; $C\mathit{H}_3{\cdot}CH_2$; α-methylbutyrate). The integrated intensities of these bands corresponded to a mixture of acetic acid, isobutyric acid, and α-methylbutyric acid in the ratio 3 : 1 : 1.

This very simple technique does not seem to have been applied to such esters before. It is capable of giving a quantitative determination of most of the acids which are known to occur in veratrum alkaloids, and is probably the method of first choice in such cases.

Investigation is being continued of the nature of the non-volatile fraction from the hydrolysis of bussein, and of the method of attachment of the volatile acids to it.

## EXPERIMENTAL

*Direct Isolation of the Acids.*—Bussein (218 mg.) was refluxed for 2 hr. in sodium hydroxide solution (12 ml.; 0·17N in 67% aqueous methanol). The hydrolysate was adjusted to pH 2·5 with dilute sulphuric acid, and the volatile acids were steam-distilled. The distillate (40 ml.) was titrated with sodium hydroxide (0·13N) to pH 9 with a pH-meter. 7·7 ml. of alkali were required, corresponding to 3·92 mol. of volatile acid.

In another experiment, using 218·3 mg. of bussein, the solution of volatile acids obtained was made alkaline with sodium hydroxide, concentrated to 5·8 ml., adjusted to pH 6·4 with hydrochloric acid (0·1N), and refluxed for 2½ hr. with ethanol (5·8 ml.) and *p*-phenylphenacyl bromide (275·4 mg.). The hot solution was filtered, and the filtrate evaporated to dryness. The residue was taken up in water (25 ml.) and extracted with benzene (8 × 25 ml.). The benzene extract was dried and evaporated, leaving a yellow oil (213·7 mg.). This was dissolved in hexane–benzene (3 : 1; 10 ml.) and chromatographed on silicic acid–Celite (3 : 1; 6 g.). Elution with the same solvent gave an oil (6·7 mg.). Hexane–benzene (2 : 1) gave *p*-phenylphenacyl bromide, identical with an authentic sample. Hexane–benzene (1 : 1) gave two overlapping peaks. The first (7 fractions) consisted largely of *p*-phenylphenacyl α-methylbutyrate (44·0 mg.), needles, m. p. 70·5—72° (from hexane) (Found: C, 75·9; H, 6·9. Calc. for $C_{19}H_{20}O_3$: C, 77·0; H, 6·8%. $[\alpha]^{\circ}_{23} = -4°$). The second (11 fractions) consisted largely of *p*-phenylphenacyl isobutyrate (31·3 mg.), needles, m. p. 81—82° (from hexane) (Found: C, 76·0; H, 6·6. Calc. for $C_{18}H_{18}O_3$: C, 76·6; H, 6·4%). Further elution with benzene gave *p*-phenylphenacyl acetate (94·7 mg.), plates, m. p. 109—110° (from benzene) (Found: C, 75·6; H, 5·7. Calc. for $C_{16}H_{14}O_3$: C, 75·6; H, 5·55%).

*Estimation by Spectroscopy.*—Bussein (184·2 mg.) was dissolved in methanol (5 ml.), and sodium hydroxide solution (*ca.* 0·3N; 5 ml.) added. The solution was refluxed for 40 min., acidified with a volume of dilute sulphuric acid equivalent to the alkali taken (6·3 ml.; determined by titration of an independent sample of the alkali), and the solution evaporated to dryness under reduced pressure. Water (5 ml.) was then added, and the solution taken to dryness again. The combined distillates were titrated (phenolphthalein) against sodium hydroxide solution (10·4 ml. of 0·1N required; this is equivalent to 4·9 mol. of alkali per mol. of bussein). The titrated solution was evaporated to dryness, and the n.m.r. spectrum of the residue determined in deuterium oxide solution.

National Institute for Medical Research,
Mill Hill, London N.W.7.
University of Ibadan, Nigeria.

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