A GAS CHROMATOGRAPHIC SEPARATION OF THE VOLATILE FATTY ACIDS OF BLACK TEA

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The essential oil of tea can be separated from the other tea constituents by steam distillation and rectification of the aqueous distillate, or by extraction of an aqueous distillate with organic solvents such as ethyl ether. The yield of essential oil from a fully fermented black tea of average quality ranges from 0.01 to 0.02% of dried tea weight.

According to YAMAMOTO[1,2], 10 to 15% of this essential oil consists of acids. This fraction was investigated by the school of YAMAMOTO[1-3], but at a time when modern chromatographic techniques were not available. These authors showed that approximately half of the acid fraction consisted of palmitic acid. Considerable amounts of isovaleric and caproic acid were found to be present, as well as salicylic acid, which probably resulted mainly from hydrolysis of its methyl ester. Small quantities or traces of propionic acid, butyric acid, caprylic acid, benzoic acid and phenylacetic acid were detected.

In the course of a fractionation of the essential oil from black tea, we made a gas chromatographic analysis of its fatty acid content, which we report here. After a prefractionation of the acids by distillation, the lower boiling constituents were analysed as free acids on Perkin-Elmer columns BA (di-2-ethyl-hexyl sebacate + 10% sebacic acid) and A (di-isodecyl phthalate). The high boiling constituents were fractionated, after esterification with diazomethane, on Perkin-Elmer columns Q (apiezon "L" grease) and P (polydiethylene glycol succinate). Both methods were used for the fractions with intermediate boiling range. All the gas chromatograms were run at temperatures below 200°. Our analytical investigation therefore includes only the *n*-fatty acids up to C-12 and the branched chain acids of the same boiling range. No attempt was made to identify the higher boiling acids, since, owing to the method of preparation, our essential oil would only contain a small percentage of this tea acid fraction.

All the *n*-fatty acids from C-1 to C-12 could be identified in the mixture except undecylic acid. Identification was carried out by adding various test compounds to the mixture and noting the overlapping of the peaks, and by plotting the log retention time *versus* the number of carbon atoms. Formic and acetic acid could be detected as

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the free acids, capric and lauric acid as the methyl esters only, while all the intermediate acids ofthe *n*-series (except undecylic) were identified as the free acids and as the esters.

The gas chromatograms of the free acids show, in addition to the peaks caused by the *n*-acids, six further peaks. The two with the shortest retention times could be identified as isobutyric acid and isovaleric acid by the technique of overlapping. According to their retention times, peaks A, B and E can be attributed to the C-6, C-7 and C-8 *iso*-acids (Fig. 1), while peak C is caused by the presence of a non-identified acid not belonging to the *n*- or *iso*-series.

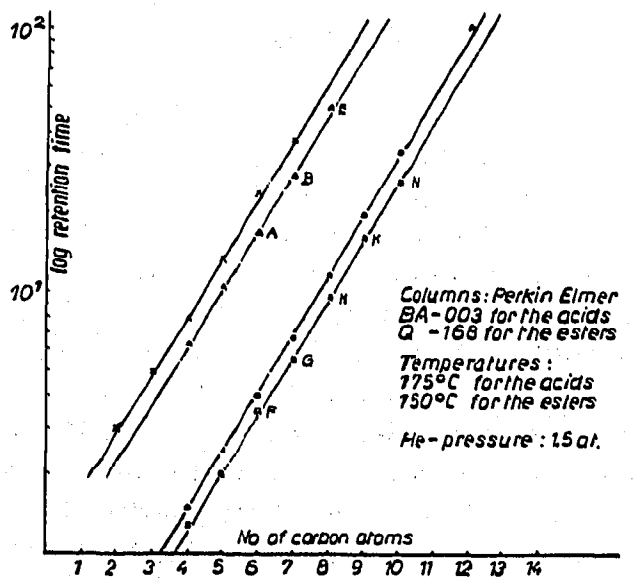

Fig. 1. Relationship between log retention time and number of carbon atoms in the molecule for fatty acids present in essential oil from black tea. (×) *n*-fatty acids; (△) *iso*-fatty acids; (○) methyl esters of *n*-fatty acids; (□) methyl esters of *iso*-fatty acids.

The gas chromatograms of the methylated acids exhibit a greater number of peaks caused by substances not belonging to the *n*-acid series. The esters of isobutyric acid and isovaleric acid could again be identified by overlapping. The plot of log retention time *versus* carbon number (Fig. 1) indicates that peaks F, G, H, K and N belong to the same *iso*-series, so that, in addition to C-6, C-7 and C-8, the presence of the branched acids C-9 and C-10 is also probable. Peaks I, L, M, O and P have not yet been identified.

It may therefore be safely assumed that all *n*-fatty acids and *iso*-fatty acids from C-1 to C-10 are present in the essential oil of black tea, along with lauric acid (*n*-C-12) and at least five other acids boiling in the same range and not belonging to the two mentioned homologous series.

Some examples of the chromatographic runs obtained are reproduced here (Figs. 2 and 3), in order to give an idea of the quantitative repartition of the various acids. We refrain from giving exact figures, because the percentages of the various acids vary considerably according to the methods of isolation and fractional distillation of the essential oil. According to the amounts in which they occur, the volatile acids (up to *n*-C-12) can be classified in four groups:

(a) between 10 and 20% of investigated acid fraction: *n*-caproic acid and isovaleric acid.

(b) between 4 and 10% of investigated acid fraction: formic acid, acetic acid, propionic acid, *n*-valeric acid, isobutyric acid, non-identified substance causing peak C.

(c) between 1 and 4% of investigated acid fraction: *n*-butyric acid, isocaproic acid, isoheptylic acid.

(d) below 1% of investigated acid fraction: *n*-heptylic acid, *n*-caprylic acid, pelargonic acid, *n*-capric acid, *n*-lauric acid, *iso*-acids C-8, C-9 and C-10, non-identified substances causing peaks I, L, M, O and P.

Of the unidentified peaks, only C is present in considerable quantity. Overlapping trials with test substances indicated that the compound causing this peak differs from lactic acid, crotonic and tiglic acid, benzoic and phenylacetic acid, and also from the phenols salicylic acid methyl ester, phenol, *o*-, *m*- and *p*-cresol, guaiacol and eugenol.

EXPERIMENTAL

(*a*) *Isolation of volatile acids*

The volatile components of a black tea infusion were separated by steam distillation and continuous extraction of the distillate with ether. The ether extract was continuously concentrated and all the evaporated solvent re-used in the extraction process in order to recover the volatiles evaporated with the ether. The resulting final concentrate of essential oil in ether was repeatedly extracted with a 0.2 *N* aequous solution of Na_2CO_3, the combined aqueous layers acidified to pH 2.5 by addition of dilute H_2SO_4 and exhaustively extracted with ether. The combined organic extracts containing the volatile acids were dried over $MgSO_4$.

In order to obtain sufficient material for our investigation, the organic extracts of several black tea batches of different origin (Ceylon, South India, Assam, Java and Kenya, total quantity approx. 150 kg) were pooled.

(*b*) *Prefractionation by distillation*

The combined dried ether extracts were concentrated, using a "Widmer" fractionating column and a very slow distillation speed. In order to remove the last few ml of ether, the water bath temperature was raised to 100°. This solvent residue, distilling at 34° to 36°, was collected as fraction 1. It contained approx. 10% of acids. Distillation was then continued at 11 mm Hg, using a small Claisen flask with "Vigreux" column. The collection flask was cooled with dry ice–acetone. Between 60° and 95°, 0.6 g of distillate was recovered as fraction 2. Another 0.4 g, fraction 3, distilled between 95° and 120°. The residue was then transferred, with the help of some ether, to a glass bulb distillation tube, the solvent evaporated and 0.05 g of acids (= fraction 4) distilled at an air bath temperature of 85° to 140°. Residue: 0.1 g.

(*c*) *Preparation of the methyl esters*

50 mg of fraction 3 or 4, distillation residue or test acid were dissolved in a few ml of dry ether and treated with a solution of diazomethane in ether at room temperature

until no further reaction occurred. The ether was evaporated and the residue distilled under reduced pressure in a glass bulb tube.

(d) Gas chromatographic separations

A Perkin-Elmer Fractometer 116 with thermistor detector, 2 m Perkin-Elmer standard columns and He as carrier gas (at 1.0 or 1.5 atm overpressure) were used.

Usually 5 mg of substance was injected for a chromatographic separation. For overlapping trials, 3 mg of each test substance was mixed with 5 mg of the fraction to be tested and 5 mg of the mixture injected.

Fraction 1 was resolved on a column BA at 150°, yielding a solvent peak, a formic and an acetic acid peak (Fig. 2).

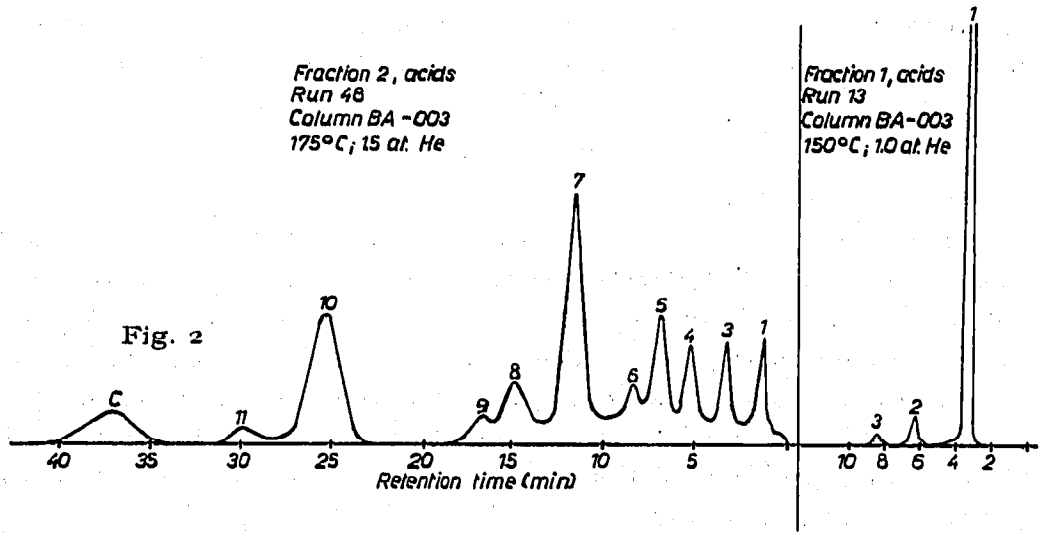

Fig. 2

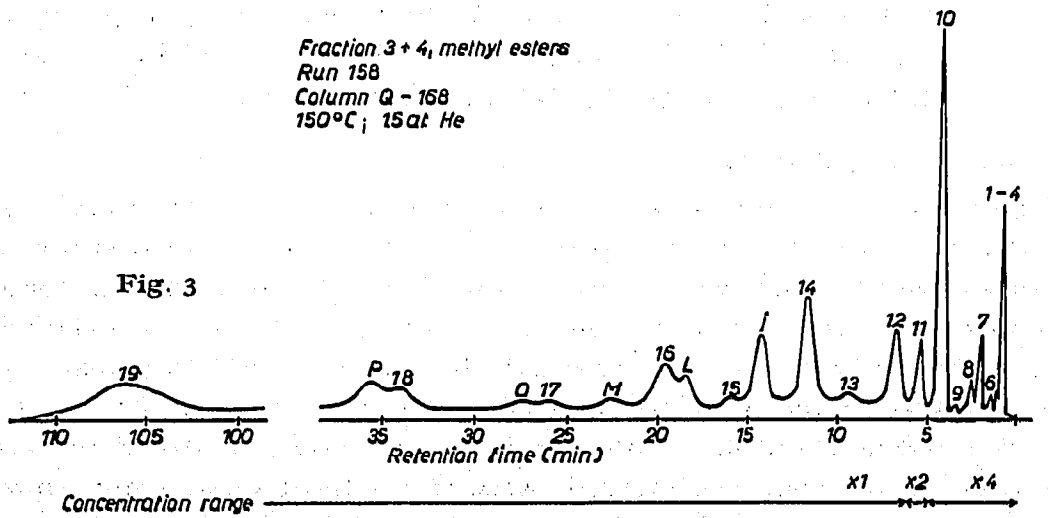

Fig. 3

Figs. 2 and 3. Examples of chromatographic runs of volatile acids of essential oil from tea, in their free and esterified forms. Peaks: 1 = solvent (mostly ether); 2 = formic acid (C-1); 3 = acetic acid (C-2); 4 = propionic acid (C-3); 5 = isobutyric acid (*iso*-C-4); 6 = *n*-butyric acid (*n*-C-4); 7 = isovaleric acid (*iso*-C-5); 8 = *n*-valeric acid (*n*-C-5); 9 = isocaproic acid (*iso*-C-6); 10 = *n*-caproic acid (*n*-C-6); 11 = isoheptylic acid (*iso*-C-7); 12 = *n*-heptylic acid (*n*-C-7); 13 = isohexylacetic acid (*iso*-C-8); 14 = caprylic acid (*n*-C-8); 15 = 7-methylcaprylic acid (*iso*-C-9); 16 = pelargonic acid (*n*-C-9); 17 = 8-methylpelargonic acid (*iso*-C-10); 18 = *n*-capric acid (*n*-C-10); 19 = lauric acid (*n*-C-12); C, I, L, M, O, P = non-identified acids not belonging to the *n*- or *iso*-series.

Fraction 2 was resolved on a column BA at 175° (Fig. 2) and a column A at 170° into all C-2 to C-7 *n*- and *iso*-acids plus the unidentified peak C.

Fractions 3 and 4 yielded, under the same conditions, 2 additional peaks, identified as C-8 *iso*- and *n*-acids. The methylated fractions 3 and 4 were resolved at 150° on a column Q, yielding the peaks reported in Fig. 3. A considerably poorer resolution was obtained on a column P at 120°.

No peaks were obtained from the methylated distillation residue on column Q at 220° and column P at 200°.

SUMMARY

The volatile acids present in the essential oil of black tea were isolated and fractionated by gas chromatography of the free acids and their methyl esters. All the *n*-fatty acids from C-1 to C-12, except undecylic acid, were found in the mixture. Isobutyric and isovaleric acid were also identified, and the presence of the *iso*-acids C-6 to C-10 of the same series was assumed from the plots of log retention time *versus* carbon number. Five or six unidentified acids not belonging to the *n*- or *iso*-fatty acid series were also present.

REFERENCES

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