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SHORT COMMUNICATION

STEAM VOLATILE ACIDS OF LATAKIA TOBACCO LEAF*

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Abstract—An acidic extract of Latakia tobacco was steam distilled, esterified and subjected to gas–liquid chromatography on surface-coated open-tubular columns. Twenty-two carboxylic acids have been identified by comparison of the retention times with those of authentic compounds.

INTRODUCTION

THE CHEMICAL constituents of Latakia, a smoke-cured Oriental tobacco, have been the subject of several papers.[1,2] The acids of Latakia leaf are a major class of compounds likely to contribute to the distinctive flavour of this tobacco. To date, some ninety carboxylic acids have been reported in the leaf and smoke from other more widely used varieties of tobacco.[3] In addition, studies[4,5] have been made on the compounds present in wood-smoke.

Gas–liquid chromatography (TLC) is a well-established method for the separation and identification of carboxylic acids in the form of their methyl esters. The efficacy of open-tubular columns in the separation of complex mixtures of fatty acid methyl esters has been previously demonstrated.[6]

RESULTS AND DISCUSSION

An acidic fraction obtained from an $NaHCO_3$ extract of the combined petrol and ether-soluble portion of Latakia tobacco was steam-distilled and, after purification and esterification, the fraction was evaporated to small bulk before analysis by GLC.

The mixture was chromatographed on three capillary columns coated with Tween 85, Silicone gum rubber (SE30) and Apiezon L. and peaks were identified by the addition of authentic compounds to the ester mixture. In order to determine whether any unsaturated compounds were present in the extract, a portion of the ester mixture was brominated by the

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dropwise addition of a solution of Br_2–CCl_4 (2 per cent v/v) until no further decolorization took place. A comparison of the chromatograms obtained by GLC analysis of the brominated mixture with the original chromatograms showed the absence of several peaks corresponding to methyl esters of unsaturated carboxylic acids. However, a distinct peak attributed to methyl crotonate was apparently unaffected by this treatment.

Analysis on the three columns yielded more than fifty peaks, twenty-five of which represented a significant concentration in the chromatogram. The peak heights in the chromatogram may not be representative of the corresponding acidic concentrations in the original leaf. Thus, several acids in tobacco leaf are known to be present in the form of their calcium salts and would therefore not be extracted with organic solvents; also nothing is known regarding the extraction efficiency of the solvents, and some of the higher acids may be assumed to be only slightly steam-volatile. However it would appear that the *n*-alkanoic and the *n*-ω-unsaturated alkenoic acids are present in relatively high concentrations, whereas the *n*-α-alkenoic acids are considerably less important. Analysis of the ether extract alone, showed a large quantity of benzoic acid.

TABLE 1. STEAM VOLATILE ACIDS DETECTED IN LATAKIA TOBACCO LEAF

Acid (as methyl ester)	SE 30	Apiezon L	Tween 85
n-Propionic	0·06	0·04	0·10
Isobutyric	0·10	0·06	0·15
n-Butyric	0·14	0·09	0·22
2-Methyl-*n*-butyric	0·21	0·14	0·28
4-Methyl-*n*-pentanoic	0·21	0·14	0·29
Crotonic	0·19	0·14	0·37
n-Pentanoic	0·29	0·21	0·39
4-Pentenoic	0·26	0·18	0·42
2-Methyl-*n*-pentanoic	0·37	0·27	0·45
2-Pentanoic	0·37	0·30	0·56
3-Methyl-*n*-pentanoic	0·41	0·32	0·49
5-Hexenoic	0·46	0·36	0·61
n-Hexanoic	0·50	0·41	0·58
2-Hexenoic	0·59	0·53	0·74
6-Heptenoic	0·72	0·67	0·83
n-Heptanoic	0·74	0·67	0·79
2-Heptenoic	0·84	0·84	0·97
Benzoic	0·92	1·04	1·21
n-Octanoic	1·00	1·00	1·00
Phenylacetic	1·10	1·19	1·39
n-Nonanoic	1·24	1·31	1·20
n-Decanoic	1·47	1·63	1·39

Retention times relative to methyl *n*-octanoate.

The methyl esters of vinylacetic, trimethylacetic, 2-methylacrylic, 2,2-dimethylbutyric, 2,3 dimethylbutyric and *tert*-butylacetic acids were added to the mixture but no peaks could be detected which corresponded to these compounds. A study of the chromatograms from the two non-polar columns revealed a series of intense peaks which appeared just before the straight-chain saturated esters. The peak which appeared prior to methyl *n*-pentanoate was unsaturated and corresponded to methyl 4-pentenoate and the corresponding compound which was eluted before methyl *n*-hexanoate was identified as methyl 5-hexenoate. A plot of the relative retention times of these unsaturated esters against C-number indicated the probable presence of the methyl-ω-alkenoates up to decenoate. In all instances these peaks appeared after the appropriate saturated ester on the Tween column.

All the straight-chain acids from acetic to hexanoic acid together with 4-methylpentanoic acid found during this investigation have previously been detected in Hickory sawdust smoke.[7] These *n*-acids and the *iso*-C_4 and *iso*-C_5 acids have also been found in cheese and butter fat.[8] 3-Methyl-*n*-pentanoic acid is regarded as an important flavour component of Turkish tobacco[9] and 2-methylbutyric acid has been detected in the aroma of cranberries.[10]

EXPERIMENTAL

Preparation of Extract

Ground Cyprus Latakia tobacco (2 kg) was extracted thoroughly with light petroleum (60–80°) in a large Soxhlet and further extracted with diethyl ether until no more pigment was removed. Both the petroleum and ethereal solutions were evaporated under slightly reduced pressure to a volume of *ca.* 2 l., combined and then extracted repeatedly with saturated aqueous $NaHCO_3$ solution. The aqueous alkaline extracts were acidified to Congo red with 2 N H_2SO_4, partitioned into ether and, after drying (Na_2SO_4) and evaporation, they yielded a combined residue which was steam-distilled.

The distillate (*ca.* 1 l.), which was collected in a cooled vessel containing aqueous KOH, was extracted successively with light petroleum and ether to remove any non-acidic material. The solution of potassium salts was evaporated to dryness on a rotary evaporator yielding a brownish residue, which was redissolved in a little water, acidified with 2 N HCl and the liberated material extracted into ether. The ethereal layer was dried and the solvent removed on a water-bath at 45°. The residue was refluxed for 10 min with a solution of BF_3–CH_3OH (approx 3 per cent w/v) to convert all acids present to the corresponding methyl esters. The resultant solution was diluted with an equal volume of distilled water, saturated with solid NaCl, and extracted twice with ether. The ethereal layer was washed repeatedly with 2 N NaOH to remove phenolic esters present in the original acidic fraction, then once with water, and finally dried with solid $CaCl_2$. The volume of the solution was reduced by careful evaporation in N_2 before injection into the chromatograph.

Preparation of Compounds

Fifteen of the identified acids were obtained from commercial suppliers and the remainder prepared by known procedures. Methyl 2-pentenoate, methyl 2-hexenoate and methyl 2-heptenoate were prepared by treating the corresponding saturated acid with dry Br_2 and PCl_3 under appropriate conditions to yield the bromo-acid, which was then esterified with CH_3OH/H_2SO_4 and dehydrohalogenated with dry quinoline.[11] Methyl 5-hexenoate and methyl 6-heptenoate were prepared by the reaction of sodio diethylmalonate with the appropriate bromoalkene. The resulting unsaturated diesters were hydrolysed, decarboxylated and esterified. Methyl 3-methylpentanoate was prepared by the above procedure using 2-bromobutane. Preparation of methyl 2-methylbutyrate was effected through a Grignard reaction on *sec*-butyl chloride followed by carbonation with solid CO_2, and esterification.

Gas Chromatography

A Perkin-Elmer F11 gas chromatograph, equipped with a flame-ionization detector, was employed throughout. The columns and conditions used for this analysis were as follows: (i) A stainless-steel open-tubular capillary column, 30 m × 0·25 mm i.d., coated with silicone-gum rubber SE-30 (SE 30). Nitrogen carrier gas inlet pressure: 10 p.s.i.g. Operating temperature: linearly programmed from 35 to 200° at 3°/min. (ii) A stainless-steel open-tubular capillary column, 50 m × 0·25 mm i.d., coated with Tween 85. Nitrogen carrier gas inlet pressure: 20 p.s.i.g. Operating temperature: linearly programmed from 35 to 180° at 2°/min. (iii) A column, similar to (ii) but coated with Apiezon L. Nitrogen carrier gas inlet pressure: 20 p.s.i.g. Operating temperature: linearly programmed from 45 to 180° at 2°/min.

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