Identification of a Low Flavor Threshold Carboxylic Acid Constituent, 4-Ethyloctanoic Acid, in Flue-Cured Virginia Tobacco

Robert C. Anderson and Alan G. Kelly*

4-Ethyloctanoic acid has been identified as a minor constituent of a flavorful acidic dichloromethane-soluble fraction of an aqueous butanol extract of flue-cured Virginia tobacco. The presence of this type of potent acidic flavorant is proposed to account for the dominant flavor quality exhibited by this tobacco fraction.

Subfractionation of a concentrate of some flavor principles of an aqueous butanol extract of flue-cured Virginia tobacco resulted in identification of some weakly flavorful acidic constituents new to tobacco (Anderson et al., 1983). It was subsequently recognized that the major characteristic flavor effect of this concentrate, however, was due to compound(s) still unknown. Further investigations have now revealed the presence in this tobacco fraction of the aliphatic carboxylic acid with the lowest odor threshold yet observed for this class of compound, viz. 4-ethyloctanoic acid (Boelens et al., 1983).

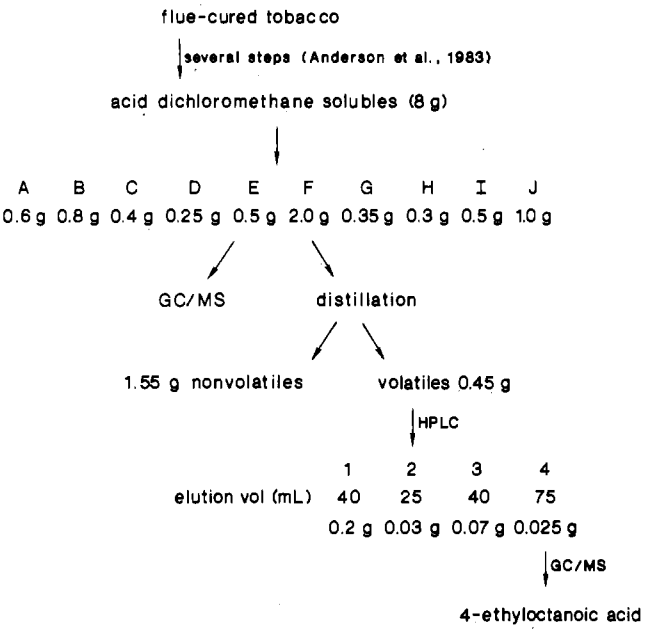
It has also been observed that the smoke flavor quality this acid produces when added to a cigarette has the same main character as that produced by the acidic tobacco fraction under investigation.

EXPERIMENTAL SECTION

Apparatus. High-performance liquid chromatography was performed with use of Waters 600A pumps coupled to a Model 660 solvent programmer with a R401 refractive index detector and Model 450 variable-wavelength UV detector coupled in tandem. Gas chromatography was performed on a Dani HR6000 capillary chromatograph with a bonded-phase 25-m SP1000 capillary column, i.d. 0.32 mm. Carrier gas was helium; flow rate, 1 mL/min. Chromatograms were analyzed on a Pye Unicam SP4270 computing integrator. GC/MS was performed using a Finnigan quadrupole mass spectrometer: EE = −70 eV; range 40–550 amu; source temperature 260 °C.

Fractionation of Tobacco. An acidic dichloromethane-soluble fraction of the aqueous butanol extract of flue-cured Virginia tobacco was obtained and further fractionated by silica gel chromatography as previously described (Anderson et al., 1983) (Scheme I). Ten fractions, A–J, were obtained. Organoleptic analysis revealed the flavor effect to reside predominantly in fraction E, with a weaker similar effect found in fraction F. Fraction E was further investigated by GC/MS analysis, but no compounds of interest were revealed. This fraction was exhausted in unsuccessful attempts to concentrate the flavor effect using HPLC fractionation alongside flavor monitoring. Thereafter, attention was turned to fraction F. Volatiles of fraction F were then obtained by bulb-to-bulb distillation [100 °C (0.5 mmHg)]. The flavor effect was found to be completely contained in the volatiles. This volatile extract was then fractionated by gradient HPLC ($MeOH/H_2O$/phosphoric acid, 50:50:1 to 90:10:1; 60 min; flow rate, 3 mL/min) using a 250 × 8 mm octadecylsilane column, to produce four subfractions. The flavor effect was found to reside in subfraction 4. This was analyzed by GC/MS, using an SP1000 capillary column with temperature programming of 100–220 °C at 4 °C/min. Injection was splitless at a temperature of 200 °C.

Scheme I. Fractionation of Flue-Cured Virginia Tobacco



Synthesis of 4-Ethyloctanoic Acid. 4-Ethyloctanoic acid was synthesized as previously described in British Patent No. 1 503 241 (1978). The acid was purified by distillation followed by semipreparative HPLC and a further bulb-to-bulb distillation. Gas chromatography indicated a purity of 99%. The mass spectrum is shown in Figure 1B.

Flavor Threshold of 4-Ethyloctanoic Acid. Flavor threshold was determined by smoking cigarettes spiked with known amounts of the flavorant. The test cigarette was hand-injected with 25 μL of an ethanolic solution of 4-ethyloctanoic acid so as to achieve an even distribution along the length of the cigarette. Blanks were prepared by injection of 25 μL of ethanol. They were then conditioned in a sealed jar overnight. Testing was done on a paired comparison basis with a blank. A series of pairs spanning 40-fold concentration range were randomly presented. The threshold was considered to be the lowest concentration detected. The flavor threshold of 4-ethyloctanoic acid thus determined was 0.6 μg/g tobacco.

RESULTS AND DISCUSSION

GC/MS analysis of the flavorful volatiles of subfraction 4 revealed a complex mixture. A comparison of the mass

Collaborative Research Unit on Plant Derived Flavours, Department of Chemistry, University of Stirling, Stirling FK9 4LA, Scotland.

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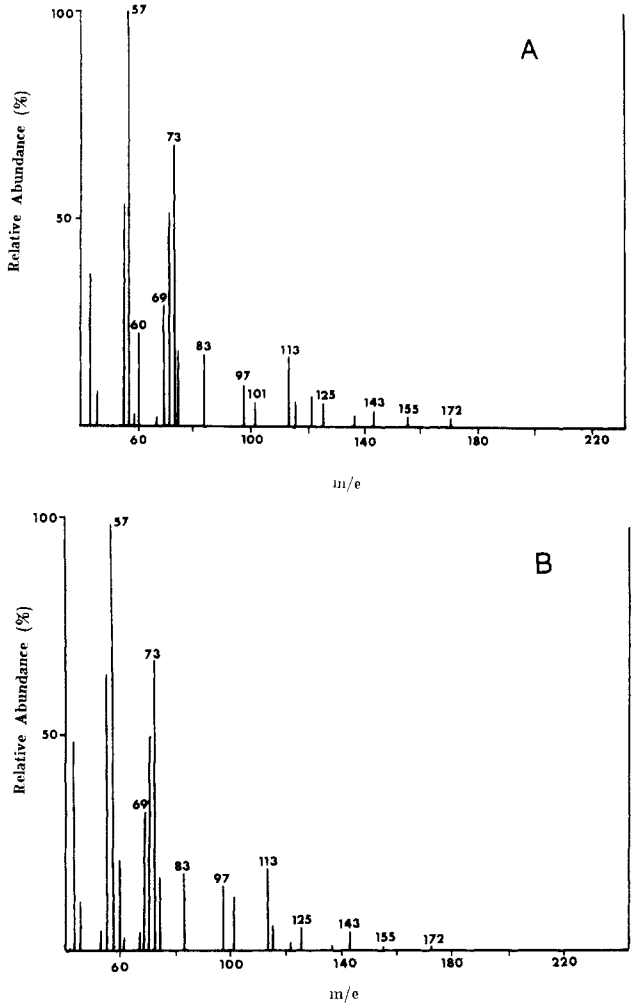

Figure 1. Mass spectra: (A) peak obtained in gas chromatographic separation of tobacco extract subfraction 4; (B) authentic 4-ethyloctanoic acid.

spectra thus obtained indicated that one of them, Figure 1A, was very similar to that of authentic 4-ethyloctanoic acid, Figure 1B. (The GC/MS examination was carried out by Gregson and Mathieson at Heriot-Watt Institute of Offshore Engineering.) Confirmation of this fact was obtained by a coinjection experiment with subfraction 4 and 4-ethyloctanoic acid, which showed the appropriate peak enhancement. It is worth mentioning here that great care was taken to avoid any possibility of cross-contamination. The tobacco extract was obtained and sealed before any 4-ethyloctanoic acid was synthesized. Likewise during GC/MS runs all operations were performed so as to eliminate any chance of cross-contamination.

The failure to find 4-ethyloctanoic acid in the more flavorful fraction E during GC/MS investigations is explicable in terms of interfering compounds masking the very low levels of 4-ethyloctanoic acid present. Only on further concentration by distillation and using HPLC was a detectable level of acid produced. The appropriate peak corresponding to 4-ethyloctanoic acid constitutes 0.1–0.3% of the total extract, and from this value an estimated tobacco concentration is 0.05–0.15 ppm. Thus, the GC/MS evidence indicates the presence of 4-ethyloctanoic acid in tobacco at a level around 0.1 ppm. This figure takes no account of probable losses occurring during HPLC subfractionation (e.g. 450-mg loading with 325-mg recovery) and is high enough to produce a weak effect on a cigarette with some panelists. A threshold of 0.6 ppm is observed by R.C.A. for 4-ethyloctanoic acid, but more sensitive panelists have a 10-fold lower threshold, i.e. below 0.1 ppm.

The identification of such a low threshold acid in tobacco has led to speculation that other similar acids may also be present and contributing at similarly low or even lower concentrations to stimulation of the "sebaceous" receptor (Anderson et al., in press) activated by 4-ethyloctanoic acid.

A study in some detail of the molecular structural requirements necessary to produce similar flavor effects has been made, and it is now clear that low threshold activity to produce such effects spreads over a group of related acidic molecules (Anderson et al., in press).

The occurrence of an aliphatic carboxylic acid with branching at C-4 has been observed in the plant kingdom with the isolation of 4-ethyloctanoic acid from costus root oil (de Rijke et al., 1978).

Registry No. 4-Ethyloctanoic acid, 16493-80-4.

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