Two New Acidic Constituents of Flue-Cured Virginia Tobacco

The aqueous butanol soluble portion of flue-cured Virginia tobacco has been fractionated to produce a flavorful acidic, dichloromethane-soluble fraction. High-performance liquid chromatography of this fraction has allowed the isolation of two compounds new to tobacco, i.e., (4-hydroxy-3-methoxyphenyl)-2-ethanol and 2-formyl-5-(ethoxymethyl)pyrrole-1-acetic acid.

The water-soluble portion of the acetone extract of flue-cured Virginia tobacco is known to be of flavor interest (Anderson and Gunn, 1977). A key fractionation step of this portion, in the isolation of associated sesquiterpene glycosides, involves a 1-butanol extraction (Anderson and Gunn, 1978). As part of an ongoing investigation of such flavorants, an aqueous butanol extract of flue-cured Virginia tobacco has been examined. In subfractionating this we have concentrated some flavor principles and isolated two compounds new to tobacco.

EXPERIMENTAL SECTION

Apparatus. High-performance liquid chromatography was performed by using a Waters 6000A pump with a R401 refractive index detector. ^1H NMR spectra was obtained on a Bruker WP80 80-MHz NMR instrument at ambient temperature in deuteriochloroform. Ultraviolet spectra were measured on a Perkin-Elmer 402 spectrometer in ethanol solution. Infrared spectra were measured on a Perkin-Elmer 577 spectrometer in chloroform solution.

Fractionation of Tobacco. Flue-cured Virginia tobacco in the form of shredded leaf (800 g) was stirred with saturated aqueous butanol for 4 days (Scheme I). The extract was then evaporated to dryness and the residue extracted with water. Nicotine and other bases were removed by passage through an IRC-50 ion-exchange column. The resultant material was fractionated between water and dichloromethane to give a flavorful dichloromethane-soluble fraction. This was separated into acidic and nonacidic portions by a sodium carbonate extraction. The acidic portion was fractionated on a silica gel column (eluant chloroform–methanol, 96:4 to 90:10) to give six subfractions F1–F6. Organoleptic analysis revealed that F2 and F3 possessed desirable flavor properties. These were therefore combined and resolved into their individual components by semipreparative HPLC. Separation was obtained on a 250 × 8 mm, C^{18} reverse-phase column using methanol–water, 30:70, as the eluant. Fractions obtained were analyzed by the usual spectroscopic means, with identifications as shown in Table I.

Synthesis of 2-Formyl-5-(ethoxymethyl)pyrrole-1-acetic Acid (2). The pyrrole acid **2** (Figure 1) was synthesized in essentially the same manner as was **3** by Olsson et al. (1978).

The additional step of etherification of the hydroxymethyl group was performed as follows. The intermediate **4** (5 g) was dissolved in diethyl phosphite (200 mL) and *p*-toluenesulfonic acid (100 mg). The solution was heated under nitrogen for 24 h at 90 °C and then cooled and poured into saturated sodium carbonate solution (1.5 L). This was extracted with ether, and the ether solution was washed with water and dried. Evaporation of the solvent gave the desired ether **5** as a crystalline solid. Recrystallization from 8% aqueous ethanol gave yellow crystals: mp 94–96 °C; ^1H NMR (80 MHz, $CDCl_3$) δ 1.3 (m, 9, CH_2CH_3), 3.47 (q, 2, J = 7 Hz, OCH_2CH_3), 6.29 (m, 4, $O_2CH_2CH_3$), 4.50 (s, 2, CH_2OEt), 4.91 (s, 2, CH_2N), 6.37 (d, 1, J = 4 Hz), 7.72 (d, 1, J = 4 Hz), 7.92 (s, 1).

Scheme I. Fractionation of Flue-Cured Tobacco

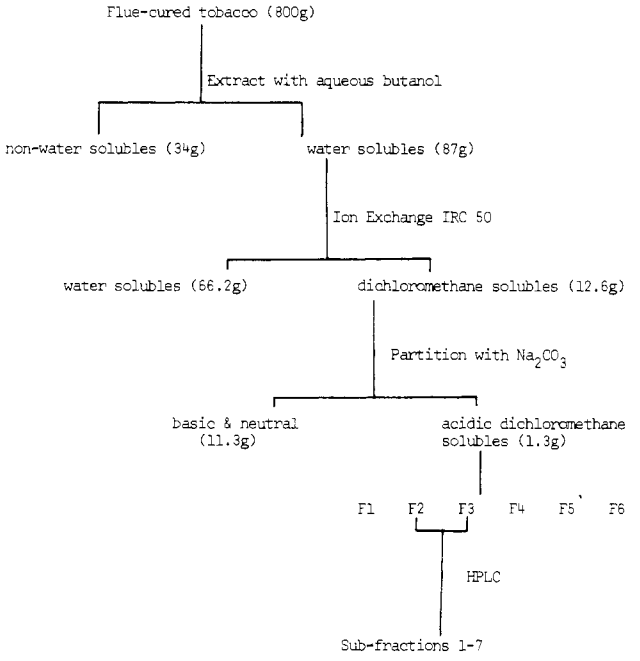

Table I. Components Isolated from F2 and F3

compound	sub-fraction	ref[a]	concn[b]
5,6-dihydro-2(1*H*)-pyridone	1	Lloyd et al. (1976)	37
(4-hydroxy-3-methoxyphenyl)-2-ethanol (1)	2		19
(*E*)-2-ethylidene-3-methylsuccinimide	3	Lloyd et al. (1976)	5
2-formyl-5-(ethoxymethyl)pyrrole-1-acetic acid (2)	4		36
phenylacetic acid	5	Stedman (1968)	46
benzoic acid	6	Stedman (1968)	36
scopoletin	7	Stedman (1968)	250

[a] In tobacco. [b] ppm dry weight of tobacco.

Base hydrolysis by the method of Olsson et al. yielded the pyrrole acid **2** as a crystalline solid. Recrystallization from toluene yielded light-colored crystals: mp 79–81 °C; ^1H NMR (80 MHz, $CDCl_3$) δ 1.22 (t, 3, J = 7 Hz, CH_2CH_3), 3.55 (q, 2, J = 7 Hz, CH_2CH_3), 4.49 (s, 2, CH_2O), 5.29 (s, 2, NCH_2), 6.23 (d, 1, J = 4 Hz, H-4), 6.87 (d, 1, J = 4 Hz, H-3), 9.5 (s, 1, CHO); λ_{max} (EtOH) 295 nm; ν_{max} ($CHCl_3$) 1705, 1660 cm^{-1}. Anal. Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.22; N, 6.63. Found: C, 56.89; H, 6.28; N, 6.56.

RESULTS AND DISCUSSION

The combined fractions F2 and F3 when subjected to HPLC separation yielded seven subfractions whose major constituents were the compounds listed in Table I.

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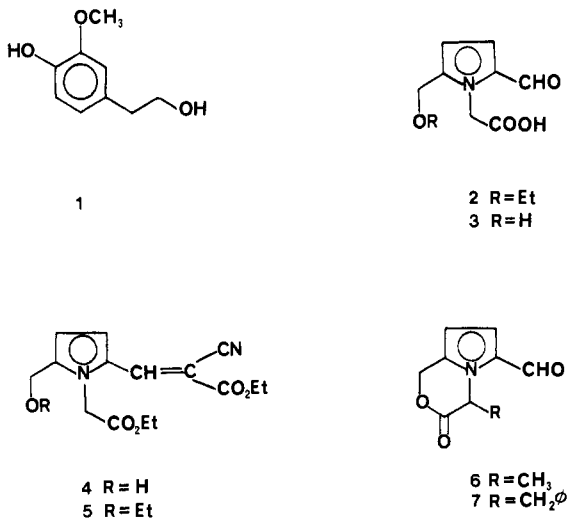

Figure 1

Identifications were based on NMR, IR, and UV spectral data and were confirmed by comparison with authentic samples and, additionally, by coinjection of these on HPLC. Of these compounds, (4-hydroxy-3-methoxyphenyl)-2-ethanol (1) and 2-formyl-5-(ethoxymethyl)-pyrrole-1-acetic acid (2) are new to tobacco. The phenol 1 has been found previously in tobacco smoke condensate (Hecht et al., 1981). The pyrrole derivative 2 is an addition to the growing number of compounds originating from the Maillard reaction of sugar and amino acids which have been found in processed foods. That this is indeed the origin of 2 seems certain as the corresponding alcohol 3 has been found in model studies of glucose–glycine reactions (Kato et al., 1977). Two related pyrrole lactones, presumably derived from glucose–alanine 6 and phenylalanine 7 reactions, have been reported in tobacco (Lloyd et al., 1976). The aroma of lactones of this type has been postulated as being of significance for the flavor of browning systems (Shigematsu et al., 1971).

The purified pyrrole acid 2 has negligible aroma; however, it does on pyrolysis produce sweet caramelic notes. The lactone derivative of 2 which we synthesized by the method of Olsson et al. has, in contrast to the related lactones isolated by Shigematsu et al. (1971), no detectable aroma. On pyrolysis it produces a weak savory/caramelic odor reminiscent of cooked food. Both 1 and 2 effected marginal improvements in the overall smoking quality of a low-tar filtered cigarette.

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Application of Carbon-13 Nuclear Magnetic Resonance to the Germination of Soybean Seeds in Vivo

The application of carbon-13 nuclear magnetic resonance to studies of intact seeds has provided a technique for direct observation and determination of chemical constituents in living matter. An extension of this technique to the behavior of soybean seeds in the presence of water allows the determination of certain chemical changes that occur during the process of germination. Observations correspond to those obtained by multistep isolation and characterization procedures, raising the possibility of registering variations in sugar and oil concentration on the same sample in a continuous fashion.

Application of carbon-13 NMR (^{13}C NMR) to the study of biological systems has shown that this powerful technique permits the direct observation and determination of chemical constituents in living matter. Recent examples involving plant cells include intact seeds (Shoolery, 1973; Schaefer and Stejskal, 1974, 1975; Schaefer et al., 1975; Kainosho, 1976; Rutar et al., 1977; Albornoz and Leon, 1980) as well as other plant tissue (Kainosho and Konishi, 1976; Kainosho and Ajisaka, 1978).

The evolution of a biological process should thus be amenable to ^{13}C NMR analysis. Indeed, it was recently shown (Chen et al., 1979) that after 2 days the spectrum

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