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SESQUITERPENOIDS FROM FLUE-CURED TOBACCO LEAVES

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Key Word Index—Flue-cured tobacco; γ-cadinene; δ-cadinene; 2-keto-α-cyperone; solavetivone; solanascone; occidol; rishitin; 2,3-dehydrosolanascone.

Abstract—Eight sesquiterpenoids were identified from an essential oil of flue-cured tobacco leaves. 2,3-Dehydrosolanascone was a new compound in nature.

INTRODUCTION

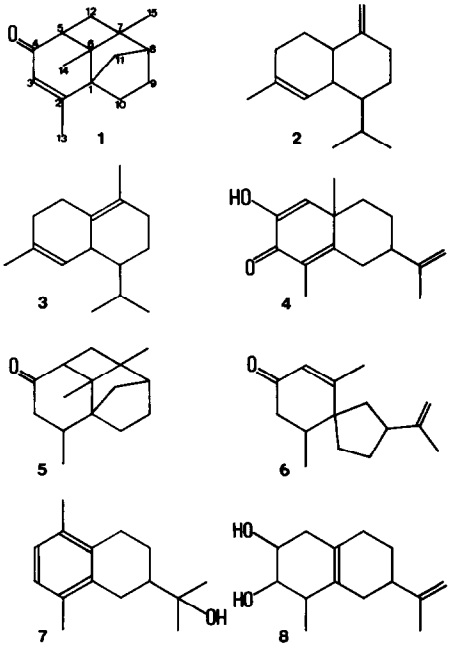
More than 800 volatile compounds are known to exist in tobacco leaves. A great many of the flavor components are presumably derived from diterpenoids, such as cembranoids and labdanoids, or from carotenoids which have been identified in several kinds of tobacco plants [1]. However, little is known about the presence of mono- and sesquiterpenoids in tobacco.

Here we report the identification of eight sesquiterpenoids from flue-cured tobacco leaves. One of them is a novel tetracyclic sesquiterpene ketone which we have named 2,3-dehydrosolanascone.

RESULTS AND DISCUSSION

A chloroform-soluble portion of flue-cured tobacco leaves was fractionated as detailed in the Experimental. Column chromatography of the neutral volatiles was carried out using hexane and diethyl ether with stepwise increases in the polarity. Each of the fractions was analysed by capillary GC and capillary GC/MS.

Compound **1** was isolated by prep. GC from the fraction eluted with hexane–20% diethyl ether. The mass spectrum of **1** gave a molecular ion peak at m/z 216 corresponding to $C_{15}H_{20}O$. The UV spectrum exhibited a maximum at 250 nm (log ε 3.53) indicating the presence of an α,β-unsaturated ketone group. This assignment was supported by a band at 1659 cm^{-1} in the IR spectrum. No absorption bands of hydroxyl groups were observed. The ^1H NMR spectrum exhibited an olefinic methyl proton (δ1.97 *d*, J = 0.12 Hz) which showed a long-range coupling with an olefinic proton (δ 5.90, *d*, J = 0.12 Hz). This was confirmed by a decoupling experiment and was consistent with the presence of a –CO–CH=C (Me)– group in a molecule. Two methyl groups (δ0.97 and 1.13) attached to quaternary carbons were observed. These data suggested that **1** was a tetracyclic sesquiterpene having an α,β-unsaturated ketone. In the ^1H NMR spectrum, signals of other methylenes and methines were superimposed between δ1.50 and 2.65. The ^{13}C NMR spectrum displayed 15 signals supporting the molecular formula $C_{15}H_{20}O$ and was similar to that of solanascone (**5**), which is a tetracyclic sesquiterpene ketone, except for signals of two olefinic carbons (δ126.6 and 165.6) and one olefinic methyl carbon (δ22.4) [2]. These results indicated the structure of a tetracyclic sesquiterpene ketone for **1**.



This structural assignment was further confirmed by synthesis. The hydrogenation of **1** will give two possible products, which are solanascone (**5**) and its epimer, by the protonation from either side of the plane of the double bond between C-2 and C-3. Actually, the platinum oxide hydrogenation of **1** (as detailed in the Experimental) exclusively produced two compounds in the ratio 43:7. The major product was identified as solanascone (**5**) by direct comparison of its retention time on capillary GC (34.2 min) and mass spectrum with authentic **5**. The minor one was considered to be 2-epi-solanascone because the mass spectrum was similar to that of **5**. Therefore, the structure of **1** was established to be the tetracyclic

sesquiterpene ketone having a double bond between C-2 and C-3. This novel compound was named 2,3-dehydrosolanascone (**1**).

The identification of γ-cadinene (**2**) and δ-cadinene (**3**) in the fraction eluted with hexane, 2-keto-α-cyperone (**4**), solanascone (**5**) and solavetivone (**6**) in the hexane–10% diethyl ether fraction, solavetivone (**6**) and occidol (**7**) in the hexane–20% diethyl ether fraction and rishitin (**8**) in the diethyl ether fraction was accomplished by comparison of retention times on GC and mass spectra with authentic materials. γ-Cadinene (**2**) is a component of citronela oil [3] and occidol (**7**) is reported as a constituent of an essential oil of *Thuja occidentalis* [4]. Rishitin (**8**) is well-known as a phytoalexin of potato [5]. However, they have not been detected previously in tobacco leaves.

EXPERIMENTAL

The MeOH extract of flue-cured tobacco leaves (4.4 kg) was dissolved in H_2O, washed with hexane, extracted with $CHCl_3$, concd, steam-distilled and then washed with 5% H_2SO_4 followed by 5% Na_2CO_3 to finally give a volatile neutral fraction (4.6 g). A part of this fraction (1.22 g) was dissolved in the minimum vol. of Et_2O, applied to a column of Si gel (60 g, Kieselgel 60, Merck) and eluted with hexane and Et_2O by stepwise increases in the polarity.

Each of the fractions eluted from the column was analysed by capillary GC (0.28 mm × 50 m glass column of OV-101, temp. programmed from 100° to 240° at 2°/min, He at 50 ml/min, FID) and capillary GC/MS (on the same column).

2,3-Dehydrosolanascone (**1**) was isolated by prep. GC as an oil (1.2 mg). The retention time of **1** on GC was 9.8 min (3 mm × 1 m packed column 5% OV-101 on Chromosorb W, temp. programmed from 120° to 240° at 5°/min, He at 60 ml/min, TCD). The following spectra were obtained. GC/MS 70 eV, *m/z* (rel. int.): 216 $[M]^+$ (29), 201 (44), 188 (72), 173 (100), 134 (39), 105 (29), 91 (45), 79 (29), 77 (27), 69 (47), 41 (35); IR ν_{max}^{film} cm^{-1}: 2979, 1659, 1379; UV λ_{max}^{EtOH} nm: 250 (log ε 3.53); ^1H NMR (300 MHz, $CDCl_3$): δ0.97 (3H, *s*), 1.13 (3H, *s*), 1.97 (3H, *d*, *J* = 0.12 Hz), 5.90 (1H, *d*, *J* = 0.12 Hz), 1.50–2.65 (10H, *m*); ^{13}C NMR (25 MHz, $CDCl_3$): δ17.9 (*q*, C-14), 17.9 (*q*, C-15), 22.4 (*q*, C-13), 24.4 (*t*, C-10), 25.8 (*t*, C-9), 36.7 (*t*, C-11), 44.3 (*d*, C-8), 44.6 (*s*, C-7), 47.0 (*d*, C-5), 47.5 (*s*, C-1), 48.4 (*t*, C-12), 53.1 (*s*, C-6), 126.6 (*d*, C-3), 165.6 (*s*, C-2). 202.1 (*s*, C-4). The carbon positions were tentatively assigned on the basis of chemical shifts and number of lines observed in the proton off-resonance decoupled spectrum.

Hydrogenation of **1**. Compound **1** (1.1 mg) was shaken for 10 min in EtOH under H_2 with 12 mg PtO_2 at room temp. After filtration and concn, the products were analysed by GC and GC/MS. The R_ts of the major product and the minor one were 34.2 min and 36.5 min (GC temp. programmed from 150° to 240° at 2°/min), respectively. The MS of the major product [70 eV, *m/z* (rel. int.): 218 $[M]^+$ (8), 190 (31), 121 (29), 120 (100), 105 (21), 41 (35)] is compatible with that of solanascone (**5**). The MS of the minor component showed peaks at *m/z*: 218 $[M]^+$ (7), 190 (45), 121 (25), 120 (100), 105 (25). The ratio of the compounds (43:7) was evaluated by integration of the peaks in the capillary GC.

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