

Tobacco Chemistry. 34. (3*E*,6*E*)-2,6-Dimethyl-10-oxo-3,6-undecadien-2-ol and (2*E*)-3-Methyl-4-oxo-2-nonen-8-ol. Two New Constituents of Greek *Nicotiana tabacum* L.

DAN BEHR, INGER WAHLBERG, TOSHIAKI NISHIDA and CURT R. ENZELL

Research Department, Swedish Tobacco Company, P. O. Box 17007, S-104 62 Stockholm, Sweden

Two new compounds were isolated from Greek *Nicotiana tabacum* L. and shown to be (3*E*,6*E*)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol and (2*E*)-3-methyl-4-oxo-2-nonen-8-ol by spectroscopic methods.

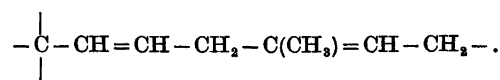
Previous studies have disclosed that tobacco contains numerous volatile, and frequently odoriferous constituents, which in all probability arise by oxidative degradation of carotenoids, diterpenoids and other isoprenoids.¹ The present communication describes the isolation and structure elucidation of two new volatile compounds from sun-cured Greek tobacco. One of these (1) clearly belongs to the group of nor-compounds derived from acyclic isoprenoids, while the biogenetic origin of the other compound (2) is uncertain.

RESULTS

Accurate mass measurements of the $[M-18]^+$ ion established that the first compound (1) had the composition $C_{13}H_{22}O_2$. Its ^{13}C NMR spectrum confirmed the presence of thirteen carbon atoms and revealed that these comprise four methyl, three sp^3 methylene, one non-protonated, oxygen-carrying sp^3 carbon, three sp^2 methine, one non-protonated sp^2 carbon and one non-conjugated carbonyl carbon. It followed from these data that the new compound must be a diene having one disubstituted and one trisubstituted double bond, a conclusion supported by the fact that the 1H NMR spectrum exhibited signals due to three

olefinic protons. Addition of $Eu(dpm)_3$ made these amenable to decoupling experiments. Thus, irradiation at the frequency of a broadened triplet, which had been shifted from δ 5.11 to 5.24, sharpened a broadened methyl singlet at δ 1.69 and a broadened two-proton doublet at δ 2.82. This indicated that the olefinic proton is allylically coupled to a methyl and a methylene group, which are attached to the same olefinic carbon. The olefinic proton, giving rise to the broadened triplet ($J = 6.5$ Hz), must be vicinal to one of the sp^3 methylene groups.

The protons of the disubstituted double bond gave rise to overlapping signals at δ 5.6, which were resolved into the AB part of an ABX_2 system, δ 6.10 (A) and 6.26 (B), on addition of $Eu(dpm)_3$. Irradiation at δ 6.10 converted the broadened two-proton doublet at δ 2.82 into a broadened singlet and, conversely, the ABX_2 system changed into an AB system when the frequency of the two-proton doublet was irradiated. This demonstrated that the carbon atoms of the disubstituted double bond are adjacent to the non-protonated carbon atom and a methylene group, respectively. Since the latter is also allylic to the trisubstituted double bond it can be concluded that the diene (1) incorporates the partial structure



Since the IR spectrum exhibited absorption at 3600 and 3550–3200 cm^{-1} , it was evident that the non-protonated carbon carries a hy-

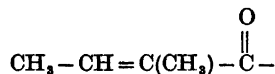
droxyl group (70.66 ppm). It also followed that the two methyl groups, giving rise to a six-proton singlet at δ 1.32 in the ^1H NMR spectrum, are attached to this carbon. Lanthanide induced shift (LIS) experiments, which demonstrated that these two methyl singlets and the signal due to one of the olefinic protons of the disubstituted double bond exhibited the largest relative shifts, 100 and 97, respectively, are in agreement with this formulation. Moreover, the largest LIS in the ^{13}C NMR spectrum of the diene *1* was observed for the signal due to the hydroxylated carbon (100) and the relative shifts of the two methyl carbon resonances were of the same magnitude as that of the sp^3 carbon signal at 139.54 ppm, 20 and 19, respectively.

The methyl ketone group, IR band at 1710 cm^{-1} , methyl singlet at δ 2.14, must be attached to the methylene group, which is adjacent to the tri-substituted double bond, *via* the remaining sp^3 methylene group, a conclusion supported by the fact that 4-oxopentanal was obtained on ozonolysis of the diene (*1*). The new tobacco constituent can therefore be formulated as 2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (*1*).

The configurations of the two double bonds in (*1*) were established as *E* on NMR evidence, *i.e.* the coupling constant between the olefinic protons of the disubstituted double bond was 16 Hz and the ^{13}C chemical shift value of the methyl group attached to the tri-substituted double bond was 16.02 ppm, which is diagnostic of *E*-configuration.^{3,4}

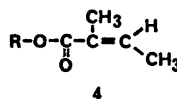
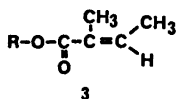
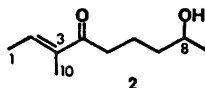
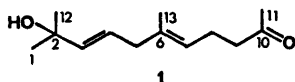
According to the ^{13}C NMR data the second compound (*2*), $\text{C}_{10}\text{H}_{18}\text{O}_3$, incorporated three methyl, three sp^3 methylene, one oxygenated sp^3 methine, one sp^3 methine, one non-protonated sp^3 carbon and one carbonyl carbon. Since the IR spectrum showed absorption at 1660 cm^{-1} and the UV spectrum had maximum

at 225 nm ($\epsilon=9600$) it was evident that the tri-substituted double bond in *2* is conjugated with the carbonyl group. As judged from the ^1H NMR spectrum, which contained a broadened three-proton singlet at δ 1.77 and a broadened three-proton doublet at δ 1.85, the two remaining substituents are methyl groups, aligned as shown in the partial structure



This was corroborated by decoupling experiments. Thus, the doublet at δ 1.85 collapsed into a singlet on irradiation at the frequency of an olefinic quartet. Conversely, irradiation at the frequency of the methyl doublet converted the quartet to a singlet. The presence of the proposed partial structure in compound *2* was further supported by the ^{13}C NMR spectrum. The signals due to the sp^3 methine and the sp^3 non-protonated carbon atoms appeared at 137.3 and 138.3 ppm, respectively, in the spectrum of the enone (*2*), which is close to the chemical shift values reported for the corresponding signals for 3-methyl-3-penten-2-one, 138.5 and 138.0 ppm.⁴ Furthermore, the ^{13}C shieldings of the methyl groups attached to the double bond (11.0 and 14.8 ppm) are close to those published for the corresponding groups in the tigloyl residue *3*, (11.9 and 14.3 ppm),⁵ but differ from those of the methyl groups in the angeloyl residue *4* (15.9 and 20.5 ppm),⁶ which indicated that enone *2* has *2E* configuration. This view was supported by the fact that the olefinic proton signal was present at δ 6.76 and 6.85 in the ^1H NMR spectra of enone *2* and the tigloyl residue *3*,⁵ respectively, but at δ 6.20 for the angeloyl residue *4*.⁶

The remaining oxygen atom in the enone *2* was accommodated by a hydroxyl substituent



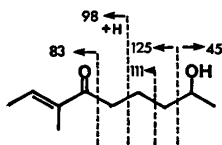


Fig. 1. MS cleavage of 2.

attached to the sp^3 methine carbon atom as shown by IR absorption at $3600\text{--}3100\text{ cm}^{-1}$ and the presence of a one-proton sextet at δ 3.77 in the ^1H NMR spectrum. Irradiation at this frequency converted a methyl doublet at δ 1.18 to a singlet. Conversely, the sextet collapsed to a triplet on irradiation at the frequency of the methyl doublet, demonstrating that the hydroxyl-bearing carbon is linked to a methyl and a methylene group. The latter must be attached to the enone moiety via the remaining two sp^3 methylene groups, which established that the new tobacco constituent (2) is 3-methyl-4-oxo-2-nonen-8-ol. Additional evidence for this formulation was obtained by the examination of the mass spectrum of 2, which contained diagnostically important peaks at m/e 170 (M), 125, 111, 98, 83 and 45 corresponding to species arising from the cleavage reactions outlined in Fig. 1.

The absolute configuration of the new tobacco isolate (2) could not be settled due to its high instability.

EXPERIMENTAL

Optical rotations were measured on a Perkin-Elmer 141 polarimeter, IR spectra on Digilab FTS-14 and Perkin-Elmer 257 instruments and UV spectra on a Varian Techtron 635 UV-visible spectrophotometer. Mass spectra were recorded on an LKB 2091 instrument and accurate mass measurements were carried out on a Varian MAT 311 instrument at the Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm.

Fourier transform ^1H NMR (100 Mz) and ^{13}C NMR (25.16 MHz) spectra were obtained in CDCl_3 solutions using TMS as internal standard and on a Varian XL-100-12 spectrometer, which was equipped with an S-124 FT and disk accessories and controlled by a Varian 620/L computer. Gas chromatography was performed on a Varian 1700 instrument using glass capillary columns (50 m \times 0.37 mm) coated with HB 5100. High performance liquid chromatography was carried out using a Waters

6000 A solvent delivery system, a U6K injector and an R-401 differential refractometer.

Isolation of (3E,6E)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (1) and (2E)-3-methyl-4-oxo-2-nonen-8-ol (2). A volatile neutral fraction (B8)⁷ of an extract obtained from 295 kg of sun-cured Greek *Nicotiana tabacum* L. was chromatographed over silica gel using a light petroleum/diethyl ether gradient. Two of the subfractions obtained were separated further by liquid chromatography using columns packed with Bondapak C18/Porasil (Waters) and μ -Porasil (Waters) to afford 32 mg of 1 and 16 mg of 2 as colourless oils.

(3E,6E)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (1) (Found: $[\text{M}-\text{H}_2\text{O}]^+$ 192.1500. Calculated for $\text{C}_{13}\text{H}_{20}\text{O}$: 192.1514) had IR bands at 3600 (m), $3550\text{--}3200$ (m), and 1710 (s) cm^{-1} ; ^1H NMR peaks at δ 1.32 (6 H, s), 1.60 (3 H, broad s), 2.14 (3 H, s), 2.18–2.58 (4 H, overlapping signals), 2.66 (2 H, broad d, J 6 Hz), 5.11 (1 H, broad t, J 6.5 Hz) and 5.53–5.64 (2 H, overlapping signals). On addition of $\text{Eu}(\text{dpm})_3$, ^1H NMR peaks at δ 1.69 (3 H, broad s), 1.99 (6 H, s), 2.22 (3 H, s), 2.25–2.70 (4 H), 2.82 (2 H, broad d, J 6 Hz), 5.24 (1 H, broad t, J 6.5 Hz), 6.10 (1 H, dt, J_1 6 Hz, J_2 16 Hz), and 6.26 (1 H, d, J 16 Hz) (AB part of an ABX_3 system). ^{13}C NMR peaks and assignments: C-1 29.85; C-2 70.66; C-3 139.54; C-4 125.01; C-5 42.31; C-6 135.17; C-7 123.49; C-8 22.52; C-9 43.65; C-10 208.80; C-11 29.92; C-12 29.85; C-13 16.02 ppm. The relative shifts of the ^{13}C NMR signals on addition of $\text{Yb}(\text{dpm})_3$ were: C-1 and C-12 20; C-2 100; C-3 19; C-4 11; C-5 5; C-6 3; C-7 3; C-8 3; C-9 3; C-10 6; C-11 3; C-13 2. The measurements were made within the linear LIS range (shift reagent/substrate ratio 0–0.5 and were normalized by arbitrarily assigning the value 100 to the carbon signal exhibiting the largest shift). MS peaks at m/e (composition, %): 210 (M, 1), 192 ($\text{C}_{13}\text{H}_{20}\text{O}$, 6), 177 ($\text{C}_{12}\text{H}_{17}\text{O}$, 3), 159 ($\text{C}_{12}\text{H}_{15}$, 4), 149 (6), 134 ($\text{C}_{11}\text{H}_{14}$, 44), 119 (C_9H_{11} , 28), 109 ($\text{C}_7\text{H}_9\text{O}$ and C_8H_{13} , 18), 107 (C_8H_{11} , 20), 95 (C_7H_9 and $\text{C}_8\text{H}_9\text{O}$, 22), 93 (C_7H_9 , 39), 85 (18), 81 (16), 79 (18), 69 (C_6H_9 , 19), 59 (18) and 43 (100).

Ozonolysis of (3E,6E)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (1). The diene (1, 5 mg), dissolved in CH_2Cl_2 (15 ml) and pyridine (0.1 ml) was treated with excess ozone at -60°C for 15 min. Zinc powder (0.1 g) and acetic acid (0.5 ml) were added and the mixture was stirred for 1 h during which the temperature was slowly raised to 20°C . The reaction mixture was filtered and the solution washed with aqueous NaHCO_3 and water. Evaporation of the solvent afforded 4-oxopentanal, which was identical to an authentic sample (GC_{RT} and GC-MS).

(2E)-3-Methyl-4-oxo-2-nonen-8-ol (2) (Found: M^+ 170.1278. Calc. for $\text{C}_{10}\text{H}_{18}\text{O}_2$: 170.1306) had IR bands at $3600\text{--}3100$ (m) and 1660 (s) cm^{-1} ; UV maximum at 225 nm

($\epsilon = 9600$); ^1H NMR peaks at δ 1.18 (3 H, d, J 6 Hz), 1.77 (3 H, broad s), 1.85 (3 H, dd, J_1 7, J_2 1 Hz), 2.68 (3 H, t, J 7 Hz), 3.77 (1 H, sextet, J 6 Hz) and 6.76 (1 H, broad q, J 7 Hz); ^{13}C NMR peaks and assignments: C-1 11.03; C-2 137.29; C-3 138.26; C-4 202.03; C-5 36.89; C-6 20.87; C-7 38.87; C-8 67.48; C-9 23.41; C-10 14.76 ppm. MS peaks at m/e (composition, %): 170 (M, $\text{C}_{10}\text{H}_{18}\text{O}_2$, 2), 152 ($\text{C}_{10}\text{H}_{16}\text{O}$, 13), 137 ($\text{C}_8\text{H}_{14}\text{O}$, 12), 125 (2), 111 ($\text{C}_7\text{H}_{11}\text{O}$, 9), 98 ($\text{C}_6\text{H}_{10}\text{O}$, 7), 83 ($\text{C}_5\text{H}_8\text{O}$, 100), 69 (5), 55 (60) and 45 (8).

Acknowledgement. We are grateful to Miss Ann-Marie Eklund for skilled technical assistance.

REFERENCES

1. Enzell, C. R., Wahlberg, I. and Aasen, A. J. *Fortschr. Chem. Org. Naturst. In press.*
2. de Haan, J. W. and van der Ven, L. J. M. *Org. Magn. Reson.* 5 (1973) 147.
3. Aasen, A. J., Nishida, T., Enzell, C. R. and Devreux, M. *Acta Chem. Scand. B* 30 (1976) 178.
4. Marr, D. H. and Stothers, J. B. *Can J. Chem.* 43 (1965) 596.
5. Zanno, P. R., Miura, I., Nakanishi, K. and Elder, D. L. *J. Am. Chem. Soc.* 97 (1975) 1975.
6. Herz, W. and Sharma, R. P. *J. Org. Chem.* 40 (1975) 3118.
7. Kimland, B., Aasen, A. J. and Enzell, C. R. *Acta Chem. Scand.* 26 (1972) 2177.

Received March 23, 1977.