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Tobacco Chemistry. 50.* (3*S*,5*R*,8*S*,9ξ)-5,8-Epoxy-6-megastigmene-3,9-diol and (3*S**,5*R**,6*R**,7*E*,9ξ)-3,6-Epoxy-7-megastigmene-5,9-diol. Two New Nor-carotenoids of Greek Tobacco

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Two new C_{13} nor-carotenoids were isolated from Greek tobacco and shown to be (3*S*, 5*R*, 8*S*, 9ξ)-5,8-epoxy-6-megastigmene-3,9-diol (*1*) and (3*S**, 5*R**, 6*R**, 7*E*, 9ξ)-3,6-epoxy-7-megastigmene-5,9-diol (*2*) by chemical and spectroscopic methods. (−)-Loliolide (*3*), (+)-isololiolide (*4*) and (3*S*, 5*R*, 6*S*, 7*E*, 9ξ)-5,6-epoxy-7-megastigmene-3,9-diol (*5*) were also obtained. The biogenesis of these compounds is discussed.

The volatile fraction isolable from tobacco contains a large number of compounds, which are likely to arise by oxidative biodegradation of carotenoids.[2] As an addition to these we now report the isolation and structure determination of two new C_{13} nor-carotenoids from sun-cured Greek tobacco.

RESULTS

The first tobacco isolate (*1*) was unstable and decomposed on exposure to air to (−)-loliolide (*3*). Since spin decoupling experiments demonstrated the presence of partial structure A, it followed that *1* is a 5,8-epoxy-6-megastigmene-3,9-diol. This conclusion was in harmony with the mass spectrum, which contained prominent peaks at *m/z* 181 ($C_{11}H_{17}O_2^+$) and 45 corresponding to fragments formed by cleavage of the 8,9 bond.

Like (−)-loliolide (*3*),[3] *1* has the 3*S*, 5*R*-configuration and the observed coupling between H−7 and H−8, ~1 Hz, suggests that the chirality at C−8 is *S*.[4] The configuration at the remaining asymmetric centre, C−9, is unsettled.

The second tobacco isolate (*2*), $C_{13}H_{22}O_3$, gave a ^{13}C NMR spectrum containing signals due to two sp^2 methine, four methyl, two sp^3 methylene, two oxygen-carrying sp^3 methine and three fully substituted sp^3 carbon atoms, two of which are oxygen-carrying. Since the ^1H NMR spectrum displayed two overlapping one-proton multiplets at δ 4.37, one of which was deshielded to δ 5.38 in the spectrum of the acetate *6*, it followed that *2* contains a −CHOH group, allocated by spin decoupling experiments to partial structure B, and an ether of structure C. Spin decoupling experiments and spin simulation extended partial structure C *via* D to the 7-oxabicyclo[2.2.1]heptane system E, in which the W shape arrangement between H_A and H_C accounts for their coupling (−2.4 Hz).[5]

Since the IR spectrum of acetate *6* had absorption at 3620 and 3490 cm^{-1}, it was evident that the remaining oxygen atom is present as a tertiary hydroxyl group. Although there are alternative ways to link this, partial structure B and the remaining three methyl groups, which all give rise to singlets in the ^1H NMR spectrum, to partial structure E, the nor-carotenoid structure (3*S**,6*R**,7*E*)-3,6-epoxy-7-megastigmene-5,9-diol appeared most plausible.

This assignment was strongly reinforced by the mass spectrum of *2*, which contained diagnostically valuable peaks at *m/z* 208, 181, 166, 125, 124 and 109 corresponding to ions generated as shown in Scheme 1.

The relative stereochemistry at C−5 was inferred from LIS experiments using $Eu(dpm)_3$

* For part 49 see Ref. 1.

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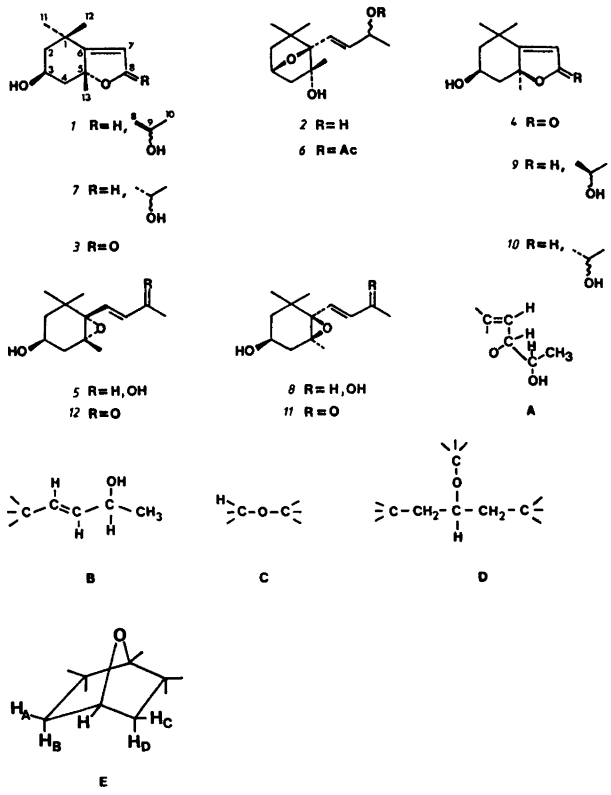
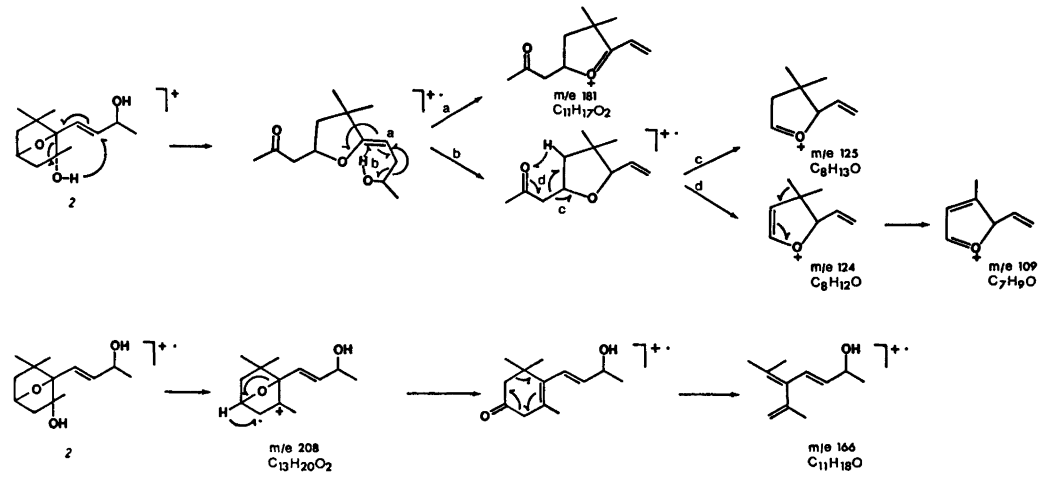
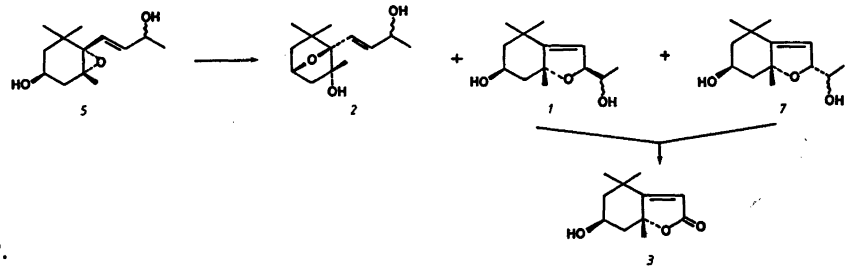


and carried out on acetate *6*. Thus, the hydroxyl group at C – 5 was the preferential complexing site and the largest shifts were found for H – 4 *endo* (100), H – 13 (85) and H – 4 *exo* (66), results which are consistent with a $5R^*$-configuration.[6]

The structure and relative stereochemistry of *2* was confirmed by a synthesis, which mimics the biogenetic route proposed in Scheme 2. Thus, treatment of (3*S*,5*R*,6*S*,7*E*,9ξ)-5,6-epoxy-7-megastigmene-3,9-diol (*5*), a compound



Scheme 1.



Scheme 2.

previously obtained from Japanese SUIFU tobacco [7] and now also isolated from Greek tobacco, with acid afforded a complex mixture, the main component of which gave ^{1}H NMR and mass spectra identical to those of *2*. Although this result is consistent with a (3*S*, 5*R*, 6*R*, 7*E*)-3,6-epoxy-7-megastigmene-3,9-diol structure of the synthetic *2*, it did not allow an assignment of absolute configuration to the naturally occurring *2*, the reason being that the optical rotation of the synthetic *2* could not be measured due to the minute quantity at hand (0.1 mg). The chirality at C−9 is undetermined in the starting compound *5* and in *2*.

Two of the minor products of the acidic rearrangement were tentatively identified as the (3*S*, 5*R*, 8*S*, 9ξ)- and (3*S*, 5*R*, 8*R*, 9ξ)-5,8-epoxy-6-megastigmene-3,9-diols (*1*, *7*) by GC−MS. They are the obvious precursors of (−)-loliolide (*3*), which is a constituent of Virginia [8] and Greek tobaccos.

By analogy, the generation of (+)-isololiolide (*4*), a compound which has now been found in Greek tobacco, would require (3*S*, 5*S*, 6*R*, 7*E*)-5,6-epoxy-7-megastigmene-3,9-diol (*8*) and the (3*S*,5*S*,8*S*)- and (3*S*,5*S*,8*R*)-5,8-epoxy-6-megastigmene-3,9-diols (*9*, *10*) as precursors. Although *8* has as yet not been found in tobacco, it is worth noting that (3*S*,5*S*,6*R*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmene-9-one (*11*), *per se* a potential precursor of (+)-isololiolide, cooccurs with the corresponding (3*S*, 5*R*, 6*S*, 7*E*)- diastereomer (*12*) in Greek tobacco [9] and that GC−MS studies indicate the possible presence of *9* and/or *10*.

EXPERIMENTAL

With the exception of accurate mass measurements, which were carried out on a Kratos MS 50−Stereo DS 50 SM/DS 50 S mass spectrometer-computer system, the instruments specified in Ref. 10 were used.

Isolation. (3*S*,5*R*,8*S*,9ξ)-5,8-Epoxy-6-megastigmene-3,9-diol (*1*, 16 mg), (3*S**, 5*R**, 6*R**, 7*E*, 9ξ)-3,6-epoxy-7-megastigmene-5,9-diol (*2*, 9 mg), (−)-loliolide (*3*, 10 mg), (+)-isololiolide (*4*, 2 mg) and (3*S*, 5*R*, 6*S*, 7*E*, 9ξ)-5,6-epoxy-7-megastigmene-3,9-diol (*5*, 64 mg) were isolated from a volatile neutral fraction (A 3) of an extract obtained from 295 kg of sun-cured Greek tobacco [11] by repeated liquid chromatography using columns packed with silica gel, Bondapak C_{18}/Porasil and μ-Bondapak CN.

(3*S*, 5*R*, 8*S*, 9ξ)-5,8-Epoxy-6-megastigmene-3,9-diol (*1*) (Found: $(M-45)^+$ 181.1208. Calc. for $C_{11}H_{17}O_2$: 181.1228) had ^{1}H NMR ($CDCl_3$) chemical shifts (δ) and assignments (mainly based on spin decoupling experiments): 1.17 (H−10, d, $J=6$ Hz), 1.18 (H−11, s), 1.32 (H−12, s), 1.60 (H−13, s), 3.57 (H−9, five lines, $J=6$ Hz), 4.21 (H−3, m), 4.53 (H−8, dd, $J=1$ and 6 Hz) and 5.31 (H−7, d, $J=1$ Hz); MS peaks at *m/z* (%, composition): 181 (59, $C_{11}H_{17}O_2$), 163 (18, $C_{11}H_{15}O$), 125 (79, $C_7H_9O_2$), 95 (29, C_6H_7O), 83 (32), 57 (100), 45 (18) and 43 (53).

1 was degraded on standing to (−)-loliolide (*3*), which had $[\alpha]_D$ −105.3° (*c* 0.2 $CHCl_3$) (reported $[\alpha]_D$ −97.2°);[3] the IR and ^{1}H NMR data agreed with those published for an authentic sample.[12]

(3*S**,5*R**,6*R**,7*E*,9ξ)-3,6-Epoxy-7-megastigmene-5,9-diol (*2*) had m.p. 44−47°, $[\alpha]_D$ −3.1° (*c* 0.6 $CHCl_3$) (Found: $[M-18]^{\cdot+}$ 208.1447. Calc. for $C_{13}H_{20}O_2$: 208.1464); IR ($CHCl_3$) bands at 3610 and 3450 cm^{-1}; ^{1}H NMR ($CDCl_3$) chemical shifts (δ) and assignments (mainly based on spin decoupling experiments): 0.88 (H−12, s), 1.23 (H−13, s), 1.30 (H−10, d, $J=6.5$ Hz), 1.41 (H−11, s), 4.37 (H−3, H−9 overlapping multiplets), 5.68 (H−7, d, $J=16$ Hz) and 5.84 (H−8, dd, $J=6$ and 16 Hz); ^{13}C NMR ($CDCl_3$) chemical shifts (δ) and assignments: C−1 43.4; C−2/C−4 48.4/47.5; C−3 75.4; C−5 81.7; C−6 91.0; C−7 134.8; C−8 123.4; C−9 68.4; C−10 23.7; C−11 25.6; C−12/C−13 32.1/31.3; MS peaks at *m/z* (%, composition): 208 (M−18, 62, $C_{13}H_{20}O_2$), 181 (5, $C_{11}H_{17}O_2$), 166 (6, $C_{11}H_{18}O$), 152 (11, $C_9H_{12}O_2$), 142 (7, $C_8H_{14}O_2$), 125 (43, $C_8H_{13}O$), 124 (10, $C_8H_{12}O$), 109 (41, C_7H_9O), 99 (16, $C_6H_{11}O$ and $C_5H_7O_2$), 82 (25, C_5H_6O and C_6H_{10}), 71 (21, C_4H_7O), 55 (13, C_4H_7 and C_3H_3O) and 43 (100).

(−)-Loliolide (*3*) had m.p. 150−151.5 °C, (reported 151.5−153 °C)[3] and $[\alpha]_D$ −85.2° (*c* 0.6 $CHCl_3$); ^{13}C NMR ($CDCl_3$) chemical shifts (δ) and assignments: C−1 36.0; C−2/C−4 45.6/47.3; C−3 66.6; C−5 87.2; C−6 183.2; C−7 112.7; C−8 172.3; C−11 30.7 and C−12/C−13 26.5/27.0; MS peaks at *m/z* (%): 196 (10), 178 (42), 163 (18), 153 (15), 140 (38), 135 (25), 125 (8), 111 (81), 95 (26), 85 (26), 67 (25), 57 (31) and 43 (100).

(+)-Isololiolide (*4*) had m.p. 117−120 °C and $[\alpha]_D$ +47.3° (*c* 0.2 $CHCl_3$) (reported m.p. 122−123 °C[13] and $[\alpha]_D$ +80.6°[3]). The IR and ^1H NMR data agreed with those published for an authentic sample.[12] ^{13}C NMR ($CDCl_3$) chemical shifts (δ) and assignments: C−1 35.1; C−2/C−4 47.8/49.7; C−3 64.7; C−5 87.0; C−6 181.6; C−7 113.0; C−8 172.0; C−11 29.9 and C−12/C−13 25.0/25.5; MS peaks at *m/z* (%): 196 (2), 178 (71), 163 (33), 153 (12), 140 (30), 135 (25), 125 (7), 111 (68), 95 (27), 81 (22), 67 (23), 57 (29) and 43 (100).

(3*S*, 5*R*, 6*S*, 7*E*, 9ξ)-5,6-Epoxy-7-megastigmene-3,9-diol (*5*) had m.p. 109−109.5 °C; $[\alpha]_D$ −110.7° (*c* 0.6 $CHCl_3$) (reported −77.3° (MeOH)).[7] The IR, ^1H NMR and mass spectra were identical to those published for an authentic sample;[7] ^{13}C NMR ($CDCl_3$) chemical shifts (δ) and assignments: C−1 34.9; C−2 47.0; C−3 63.8; C−4 40.8; C−5 66.7; C−6 69.7; C−7 124.6; C−8 137.9; C−9 67.9; C−10 23.6; C−11/C−12 24.7/29.6 and C−13 19.9.

Preparation of (3S, 5R*, 6R*, 7E, 9ξ)3,6-epoxy-9-acetoxy-7-megastigmen-5-ol (6).* Acetylation using standard conditions converted *2* into (3*S**, 5*R**, 6*R**, 7*E*, 9ξ)-3,6-epoxy-9-acetoxy-7-megastigmen-5-ol (*6*), which had IR bands at 3620, 3490, 1740 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$) chemical shifts (δ) and assignments (based on spin decoupling and spin simulation experiments): 0.86 (H−12, s), 1.20 (H−13, s), 1.33 (H−10, d, J=6.5 Hz), 1.40 (H−11, s), 1.60 (H−2 *endo*, d, J=−11.5 Hz), 1.66 (H−4 *endo*, d, J=−12.0 Hz), 1.81 (H−2 *exo*, eight lines, J=−11.5, 5.8 and −2.4 Hz), 2.01 (H−4 *exo*, eight lines, J=−12.0, 6.4 and −2.4 Hz), 2.04 ($OCOCH_3$, s), 4.36 (H−3, dd, J=6.4 and 5.8 Hz), 5.38 (H−9, m), 5.72 (H−8, dd, J=6 and 16 Hz) and 5.79 (H−8, d, J=16 Hz); relative shifts on addition of $Eu(dpm)_3$ (the measurements were made within the linear LIS range and were normalized by arbitrarily assigning the value 100 to the proton signal exhibiting the largest shift): H−4 *endo* 100; H−13 85; H−4 *exo* 66; H−11 57; H−2 *endo* 41; H−3 38; H−2 *exo* 33 and H−12 33; MS peaks at *m/z* (%): 208 (M−60, 100), 190 (11), 166 (13), 151 (11), 135 (12), 125 (70), 109 (36), 82 (31), 69 (8), 55 (9) and 43 (56).

Treatment of (3S, 5R, 6S, 7E, 9ξ)-5,6-epoxy-7-megastigmene-3,9-diol (5) with acid. To a solution of 4.8 mg of *5* in 2 ml of dioxane/H_2O (2:1) was added 5 drops of aqueous H_2SO_4 (5 %). The solution was kept at room temperature for 20 h. Dilution with water, extraction with ether and evaporation afforded a complex mixture, which was examined by GC−MS. Two of the minor products, which gave mass spectra identical to that of *1*, were tentatively identified as the (3*S*, 5*R*, 8*S*, 9ξ)- and (3*S*, 5*R*, 8R, 9ξ)-5,8-epoxy-6-megastigmene-3,9-diols (*1*, *7*). The main product, (3*S*, 5*R*, 6*R*, 7*E*, 9ξ)-3,6-epoxy-7-megastigmene-5,9-diol, was isolated (0.1 mg) from the mixture by HPLC using a column packed with μ-Bondapak CN. Its ^1H NMR and mass spectra were identical to that of tobacco constituent *2*.

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