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Tobacco Chemistry. 47. (3*S*,6*R*,7*E*,9*R*)- and (3*S**,6*R**,7*E*,9*S**)-4,7-Megastigmadiene-3,9-diol. Two New Nor-carotenoids of Greek Tobacco

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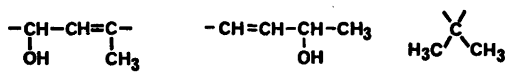
Two new C_{13} nor-carotenoids were isolated from Greek *Nicotiana tabacum* L. and shown by spectroscopic methods and synthesis to be the (3*S*,6*R*,7*E*,9*R*)- and (3*S**,6*R**,7*E*,9*S**)-4,7-megastigmadiene-3,9-diols. The sample of "3-oxo-α-ionol", previously isolated from Greek tobacco, was reinvestigated and found to contain (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one as the major component and the corresponding, partially racemic (6*R*,7*E*,9*S*)-isomer as the minor component.

Recent studies have revealed that the flavour fractions isolable from tobacco contain remarkable quantities of nori-soprenoids, which are generated by biodegradation of diterpenoids, carotenoids and higher isoprenoids.[1] We now report the structure determination of two new diastereomeric C_{13} nor-carotenoids (*1*, *2*) isolated from sun-cured Greek tobacco.

RESULTS

As shown by the IR, ^{1}H NMR and ^{13}C NMR spectra, the first compound (*1*), $C_{13}H_{22}O_2$, incorporates one di- and one trisubstituted double bond, three sp^3 methine carbons, two of which are carrying hydroxyl groups, four methyls, one sp^3 methylene, and one fully substituted sp^3 carbon atom. Spin decoupling experiments aligned the hydroxyl-carrying carbons, the double bonds and two of the methyl groups as illustrated by partial structures A and B. The remaining two methyl groups, giving rise to singlets at δ 0.86 and 0.93 in the ^{1}H NMR spectrum, were evidently attached to the fully-substituted sp^3 carbon atom, *i.e.* diol *1* includes partial structure C.



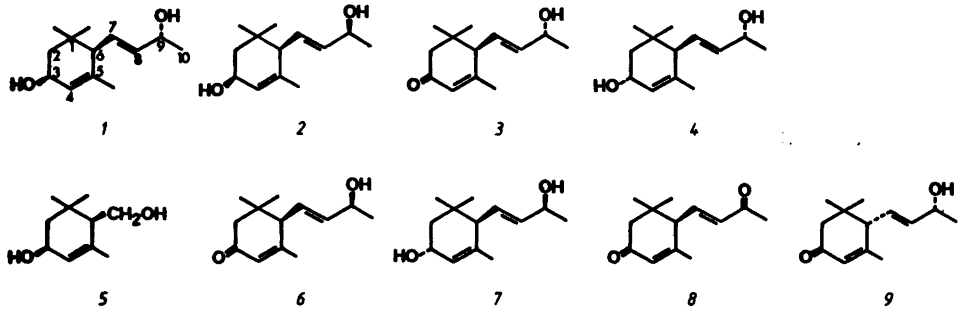
A B C

It followed from these results that the new diol is monocyclic and the nor-carotenoid structure *1*, 4,7-megastigmadiene-3,9-diol,* appeared plausible. In accordance with this structure, the mass spectrum of diol *1* exhibits a prominent peak at *m/e* 154, which corresponds to an ion formed by a retro Diels-Alder fragmentation.

Conclusive evidence was obtained by chemical means. Reduction of (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one (*3*) (*vide infra*) using $LiAlH_4$ yielded two diastereomeric diols (*1*, *4*). The more polar of these was identical in all respects to the new tobacco diol (*1*), a result which settled the structure and the 6*R*,9*R*-configuration but left the chirality at C-3 to be determined.

Selective ozonolytic cleavage of the 7,8 double-bond in diol *1* followed by reduction with $NaBH_4$ gave a product, whose mass and ^{1}H NMR spectra were identical to those of (1*S**,4*R**)-4-hydroxymethyl-3,5,5-trimethyl-2-cyclohexen-1-ol (*5*).[3] The new tobacco diol (*1*) could therefore be formulated as (3*S*,6*R*,7*E*,9*R*)-4,7-megastigmadiene-3,9-diol, while the less

* For nomenclature see Ref. 2.



1 *2* *3* *4*

5 *6* *7* *8* *9*

polar synthetic diol (*4*) is the corresponding 3*R*-epimer.

The shielding of C-2 in the ^{13}C NMR spectra of (3*S**,6*R**,7*E*)- and (3*R**,6*R**,7*E*)-4,7-megastigmadiene-3,9-diol has recently been reported to be diagnostic of the configuration at C-3, *i.e.*, the C-2 signal was present at δ 41.3 for the (3*S**,6*R**)-diol and at δ 45.5 for the (3*R**,6*R**)-diol.[4] When allowing for a systematic shift difference, our ^{13}C NMR data for diols *1* and *4* concur with these results.

The second tobacco isolate (*2*) gave a ^1H NMR spectrum containing signals assigned to two hydroxyl-carrying methine groups, one of which was adjacent to a methyl group, two methyl groups attached to (a) fully substituted carbon(s), a methyl group attached to the fully substituted carbon of a trisubstituted double bond and two protons of a disubstituted double bond. Its properties are thus markedly similar to those of the (3*S*,6*R*,7*E*,9*R*)- and (3*R*,6*R*,7*E*,9*R*)-4,7-megastigmadiene-3,9-diols (*1*, *4*) and since the mass spectra of all three compounds (*1*, *2*, *4*) were virtually identical, it seemed likely that diol *2* was a stereoisomer of diols *1* and *4*.

In order to confirm this, partially racemic (6*R*,7*E*,9*S*)-9-hydroxy-4,7-megastigmadien-3-one (*6*; *vide infra*) was reduced to the two 3,9-diols (*2*, *7*). The more polar of these gave IR, ^1H NMR and mass spectra identical to those of diol *2*. However, since the tobacco diol *2* was isolated in a minute quantity, which did not allow measurement and comparison of optical rotations, this result only showed that diol *2* is a (7*E*)-4,7-megastigmadiene-3,9-diol with the relative stereochemistry 6*R**,9*S**.

A clue to the stereochemistry at C-3 was obtained by a comparison of the ^1H NMR spectra of diols *2* and *7* with those of diols *1* and *4*. The signals due to the methyl groups at C-1 appeared at δ 0.85 and 0.93 for diol *2* and at δ 0.86 and 0.93 for the (3*S*,6*R*,9*R*)-diol (*1*), while the corresponding signals were present at δ 0.85 and 1.01 for both the (3*R*,6*R*,9*R*)-diol (*4*) and diol *7*. Moreover, the olefinic protons in diols *1* and *2* gave rise to almost identical signal patterns and these differed considerably from those displayed by the olefinic protons in diols *4* and *7*. Hence, tobacco diol *2* was assigned the structure (3*S**,6*R**,7*E*,9*S**)-4,7-megastigmadiene-3,9-diol. The less polar synthetic diol *7* has the 3*R*-configuration.

The chemical correlations described above required the (6*R*,7*E*,9*R*)- and (6*R*,7*E*,9*S*)-9-hydroxy-4,7-megastigmadien-3-ones (*3*, *6*) as starting materials. To this end a sample, previously obtained from Greek tobacco and assumed to contain these two isomers (*3*, *6*; ratio 4:1) and possibly a minor amount of a 6*S*-isomer,[5,6] was reinvestigated. Thus, high performance liquid chromatography on a microparticulate silica gel column separated it into two fractions, the less polar of which consisted of (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one (*3*). The optical rotation of this ketol (*3*) was considerably larger (+269°) than that previously reported (+177°).[5] Similarly, (6*R*,7*E*)-4,7-megastigmadiene-3,9-dione (*8*), obtained from ketol *3* by oxidation, had a larger optical rotation (+350°) than that previously reported (+293°).[5]

The more polar fraction gave IR, mass, ^1H and ^{13}C NMR spectra, which differed in minor details only from those of the (6*R*,7*E*,9*R*)-ketol (*3*). It was converted by oxidation to a product having IR, ^1H NMR and mass spectra identical to those of (6*R*,7*E*)-4,7-megastigmadiene-3,9-dione (*8*). The optical rotation of this product

(+127°), however, was lower than that of the (6*R*,7*E*)-diketone (*8*) (+350°).[5] Since the two oxidations were carried out under identical conditions, which would make contributions from epimerization at C-6 equal, this result indicated that the polar ketol fraction and the oxidation product derived thereof were partially racemic. It followed that the ketol fraction consisted of some 70 % of (6*R*,7*E*,9*S*)-9-hydroxy-4,7-megastigmadien-3-one (*6*) and some 30 % of the (6*S*,7*E*,9*R*)-isomer (*9*).

EXPERIMENTAL

For instrumental details: see Ref. 7.

Isolation. (3*S*,6*R*,7*E*,9*R*)-4,7-Megastigmadiene-3,9-diol (*1*, 9.7 mg) was isolated from a volatile neutral fraction (B 10) [8] of an extract obtained from 295 kg of sun-cured Greek tobacco by repeated liquid chromatography on columns packed with silica gel and Bondapak C_{18}/Porasil. Diol *1* was obtained as an oil, which had $[\alpha]_D$ +272° (*c* 0.29, $CHCl_3$) (Found: $[M-18]^+$ 192.1509; Calc. for $C_{13}H_{20}O$: 192.1514); IR (film) bands at 3350 and 1665 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (3 H, s), 0.93 (3 H, s), 1.27 (3 H, d, $J=6.5$ Hz), 1.64 (3 H, m), 4.0–4.5 (2 H, overlapping signals) and 5.3–5.7 (3 H, overlapping signals); irradiation at the frequency of the methyl doublet at δ 1.27 gave a doublet at δ 4.30 and, conversely, irradiation at the frequency of the signals at δ 4.30 converted the doublet at δ 1.27 to a singlet and affected the overlapping signals in the olefinic region, δ 5.3–5.7. The latter signals were also affected when the frequency of the methyl multiplet at δ 1.64 was irradiated. Conversely, irradiation at the frequency of the olefinic signals at δ 5.45 sharpened the multiplet at δ 1.64 and changed the signal pattern at δ 4.0–4.5; ^{13}C NMR ($CDCl_3$): δ 22.5 (q), 23.5 (q), 27.0 (q), 29.2 (q), 34.3 (s), 40.5 (t), 54.0 (d), 66.4 (d), 68.3 (d), 125.0 (d), 130.0 (d), 136.4 (d) and 137.1 (s) (s = singlet, d = doublet, t = triplet and q = quartet refer to the single frequency off-resonance decoupled spectrum); MS [*m/e* (%, composition)]: 192 (M–18, 25), 177 (6), 174 (5), 159 (23), 154 (25, $C_9H_{14}O_2$), 136 (32, $C_{10}H_{16}$ and $C_9H_{12}O$), 122 (55, C_9H_{14}), 109 (62), 108 (98, C_7H_8O and C_8H_{12}), 107 (55), 93 (67, C_7H_9), 69 (38), 55 (32) and 43 (100).

(3*S**,6*R**,7*E*,9*S**)-4,7-Megastigmadiene-3,9-diol (*2*, 0.9 mg) was isolated from a volatile neutral fraction (A 3) [8] obtained from the extract of Greek tobacco by repeated liquid chromatography using columns packed with silica gel, Bondapak C_{18}/Porasil and μ Bondapak C_{18}. Diol *2* was isolated as an oil (Found: $[M-18]^+$ 192; Calc for $C_{13}H_{20}O$: 192), which had IR bands at 3330 and 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (3 H, s), 0.93 (3 H, s), 1.28 (3 H, d, $J=6.5$ Hz), 1.66 (3 H, m), 4.1–4.5 (2 H, overlapping signals) and 5.3–5.7 (3 H, overlapping signals); MS [*m/e* (%)]: 192 (M–18, 18), 177 (6), 174 (9), 159 (33), 154 (18), 136 (28), 119 (62), 109 (63), 108 (87), 107 (71), 93 (81), 69 (38), 55 (33) and 43 (100).

Preparation of (3S,6R,7E,9R)-4,7-megastigmadiene-3,9-diol (1) and its 3R-isomer (4). To a solution of 72 mg of (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one (*3*) in ether was added 50 mg of $LiAlH_4$ and the reaction mixture was stirred at room temperature for 3.5 h. Work-up and chromatography on silica gel using ethyl acetate/hexane (60:40) as an eluent furnished two compounds, the more polar of which, (3*S*,6*R*,7*E*,9*R*)-4,7-megastigmadiene-3,9-diol (31 mg), was indistinguishable ($[\alpha]_D$, IR, ^1H and ^{13}C NMR, MS) from tobacco diol *1*. The less polar compound, (3*R*,6*R*,7*E*,9*R*)-4,7-megastigmadiene-3,9-diol (*4*, 15 mg), was isolated as an oil and had $[\alpha_D]$ +197° (*c* 1.54, $CHCl_3$); IR (film) bands at 3350 and 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (3 H, s), 1.01 (3 H, s), 1.28 (3 H, d, $J=6.5$ Hz), 1.62 (3 H, m), 4.1–4.5 (2 H, overlapping signals) and 5.2–5.8 (3 H, overlapping signals); ^{13}C NMR ($CDCl_3$): δ 22.6 (q), 23.6 (q), 23.9 (q), 29.4 (q), 33.5 (s), 44.6 (t), 54.0 (d), 65.6 (d), 68.5 (d), 124.9 (d), 128.9 (d), 137.1 (s) and 137.7 (d); MS [*m/e* (%)]: 192 (M-18, 28), 177 (4), 174 (5), 159 (19), 154 (21), 136 (68), 121 (38), 119 (36), 109 (48), 108 (83), 107 (30), 93 (61), 69 (35), 55 (26) and 43 (100).

Ozonolytic degradation of (3S,6R,7E,9R)-4,7-megastigmadiene-3,9-diol (1). A cold (−20 °C) solution of 20 mg of (3*S*,6*R*,7*E*,9*R*)-4,7-megastigmadiene-3,9-diol (*1*) in MeOH was treated with ozone for 10 min. $NaBH_4$ (95 mg) was added and the mixture was left at room temperature for 1 h. Work-up and chromatography on silica gel using ethyl acetate/hexane (60:40) as an eluent gave 2.1 mg of an oil (*5*), whose ^1H NMR spectrum was identical to that of (1*S**,4*R**)-4-hydroxymethyl-3,5,5-trimethyl-2-cyclohexen-1-ol (*cis*-3-hydroxy-α-cyclogeraniol).[3] Diol *5* had MS [*m/e* (%)]: 170 (M, 5), 155 (48), 137 (72), 122 (35), 109 (62), 107 (100), 96 (48), 84 (62), 69 (84), 55 (37) and 43 (82).

Acetylation of diol *5* furnished a diacetate, (1*S*,4*R*)-4-acetoxymethyl-3,5,5-trimethyl-2-cyclohexen-1-yl acetate, whose ^1H NMR spectrum differed from that of the diacetate of *trans*-3-hydroxy-α-cyclogeraniol [9] and displayed peaks ($CDCl_3$) at δ 0.98 (3 H, s), 1.01 (3 H, s), 1.80 (3 H, m), 2.04 (3 H, s), 2.05 (3 H, s), 4.09 (1 H, dd, $J=3$ and 12 Hz) and 4.30 (1 H, dd, $J=5.5$ and 12 Hz) (AB part of an ABX system), 5.30 (1 H, m, $W_{\frac{1}{2}}=20$ Hz) and 5.50 (1 H, m, $W_{\frac{1}{2}}=5$ Hz).

Preparation of (3S,6R,7E,9S)-4,7-megastigmadiene-3,9-diol (2) and its 3R-isomer (7). To a solution of 7.4 mg of partially racemic (6*R*,7*E*,9*S*)-9-hydroxy-4,7-megastigmadien-3-one (*6*) in ether was added 2 mg of $LiAlH_4$. The reaction mixture was stirred at room tempera-

ture for 1 h. Work-up and chromatography on silica gel using ethyl acetate/hexane (60:40) as an eluent afforded two diols as oils. The more polar diol, (3*S*,6*R*,7*E*,9*S*)-4,7-megastigmadiene-3,9-diol (2.3 mg), was identical (IR, ^{1}H NMR and MS) to tobacco diol *2*. The less polar diol, (3*R*,6*R*,7*E*,9*S*)-4,7-megastigmadiene-3,9-diol (*7*, 1.2 mg) displayed ^{1}H NMR ($CDCl_3$) peaks at δ 0.85 (3 H, s), 1.01 (3 H, s), 1.29 (3 H, d, J = 6.5 Hz), 1.62 (3 H, m), 4.1 – 4.5 (2 H, overlapping signals) and 5.2 – 5.7 (3 H, overlapping signals); MS [*m/e* (%)]: 192 (M-18, 17), 177 (4), 174 (2), 159 (8), 154 (18), 136 (65), 121 (37), 109 (43), 108 (80), 107 (26), 93 (61), 69 (35), 55 (28) and 43 (100).

Separation of "3-oxo-α-ionol isomers". A sample, previously obtained from Greek tobacco and reported to contain (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one as the main component,[5] was separated by recycling chromatography on a column (9 mm × 50 cm) packed with Partisil 10 into 90 mg of (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one (*3*) and 19 mg of the partially racemic (6*R*,7*E*,9*S*)-isomer (*6*). Ketol *3* was obtained as an oil and had $[\alpha]_D$ +269° (*c* 1.29, $CHCl_3$) (reported +177°);[5] ^{13}C NMR ($CDCl_3$): δ 23.6 (2 q), 27.0 (q), 27.8 (q), 36.1 (s), 47.4 (t), 55.4 (d), 67.8 (d), 125.5 (d), 125.9 (d), 138.9 (d), 163.0 (s) and 199.7 (s); the IR and ^{1}H NMR data agreed with those previously published.[5]

Ketol *6* was an oil and had $[\alpha]_D$ +69° (*c* 1.14, $CHCl_3$); IR (film) bands at 3400 and 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.96 (3 H, s), 1.03 (3 H, s), 1.30 (3 H, d, J = 6.3 Hz), 1.91 (3 H, d, J = 1.1 Hz), 4.36 (1 H, m) and 5.3 – 6.0 (3 H, overlapping signals); ^{13}C NMR ($CDCl_3$): δ 23.6 (2 q),27.1 (q), 27.8 (q), 36.2 (s), 47.4 (t), 55.5 (d), 68.0 (d), 125.5 (d), 126.2 (d), 138.8 (d), 163.2 (s) and 199.9 (s).

Preparation of (6R,7E)-4,7-megastigmadiene-3,9-dione (8). A. To an ethereal solution of 3.5 mg of (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one (*3*) was added 15 μl of a solution of sodium dichromate in dilute sulfuric acid.[5] After stirring for 1 h at room temperature the reaction mixture was worked up and chromatographed on silica gel to furnish 2.7 mg of (6*R*,7*E*)-4,7-megastigmadiene-3,9-dione (*8*) as an oil, which had $[\alpha]_D$ +350° (*c* 0.27, $CHCl_3$) (reported +293°).[5] B. Oxidation of the partially racemic ketol (*6*) using the same conditions as described under A gave a compound $[\alpha]_D$ +127° (*c* 0.15, $CHCl_3$), whose IR, ^{1}H NMR and mass spectra were identical to those of (6*R*,7*E*)-4,7-megastigmadiene-3,9-dione (*8*).

The homogeneities of all samples described above were checked by GC on a capillary column and, where possible, by ^{13}C NMR.

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