Tobacco Chemistry. 66.* (5*R*,6*S*,7*E*,9*S*)-7-Megastigmene-5,6,9-triol, a New Constituent of Greek Tobacco

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A new tobacco constituent has been isolated from tobacco and is shown to be (5*R*,6*S*,7*E*,9*S*)-7-megastigmene-5,6,9-triol by spectral methods, X-ray analysis of the corresponding 9*R*-epimer, and asymmetric synthesis. The biogenesis of the new compound is discussed.

Previous studies have shown that the flavour fractions isolated from tobacco are rich sources of C_{13}-compounds clearly derived via oxidative cleavage of the polyene chain of cyclic carotenoids.[2] As an addition to these we now report the isolation, structure determination and asymmetric synthesis of a new C_{13}-triol.

Results

The new compound (**1**, $C_{13}H_{24}O_3$, 1.3 mg) was isolated from sun-cured leaves of Greek tobacco. It contains a secondary hydroxy group [^{1}H NMR signal at δ 4.39 (ddq); ^{13}C NMR signal at δ 68.8 (d)], which was shown by spin-decoupling experiments to be present in partial structure A. The remaining oxygen atoms are accommodated by two tertiary hydroxy groups [^{13}C NMR signals at δ 75.0 (s) and 79.1 (s)]. Since the ^{1}H NMR spectrum also includes methyl singlets at δ 0.88, 1.06 and 1.22, it seemed most likely from a biogenetic point of view that **1** is a 7*E*-megastigmene-5,6,9-triol.§

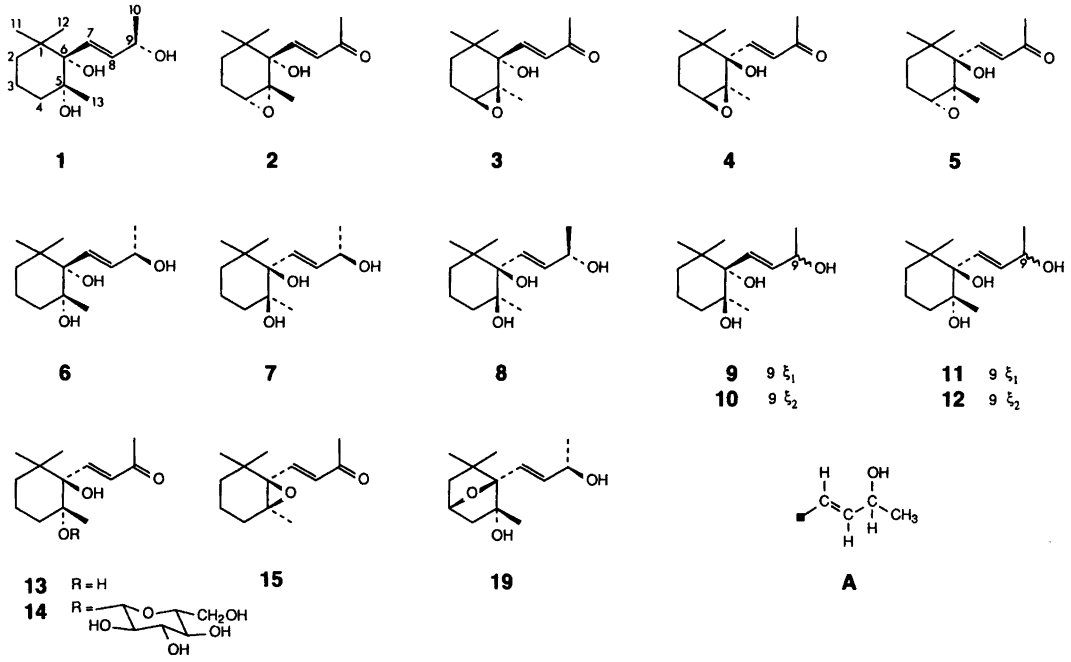
In order to obtain an insight into the biogenetic origin of **1**, it was deemed essential to determine not only the relative, but also the absolute stereochemistry. A means to achieve this has been described by Eugster *et al.*,[3,4] who have reported the asymmetric syntheses of the (4*R*,5*R*,6*S*,7*E*)- and (4*S*,5*S*,6*S*,7*E*)-4,5-epoxy-6-hydroxy-7-megastigmen-9-ones (**2** and **3**). We used their method for the preparation of these two compounds as well as the corresponding enantiomers **4** and **5**. Epoxyketol **2** was converted by reduction to the 5,6,9-triols **1** and **6**. The most polar of these proved to be identical to the new tobacco constituent **1**, demonstrating that this has 5*R*,6*S* stereochemistry but leaving the chirality of C-9 to be accounted for. Triol **6**, which in contrast to triol **1** formed single crystals, was therefore subjected to X-ray analysis.

Triol **6** formed orthorhombic crystals of space group $P4_1$. The crystal data, obtained on a Siemens/Stoe AED 2 diffractometer, were: a = 10.7881, b = 10.7881 and c = 23.7672 Å; Z = 4. The present R-value based on refinement including anisotropic thermal parameters for all non-hydrogen atoms and isotropic thermal parameters for all but the hydroxy hydrogen atoms is 0.069, further refinement being underway.[5] A stereoscopic view, which summarizes the X-ray results and demonstrates that **6** is (5*R*,6*S*,7*E*,9*R*)-7-megastigmene-5,6,9-triol, is shown in Fig. 1. As a result, triol **1** is assigned a 9*S*-configuration.

The 5*S*,6*R*,9*R*- and 5*S*,6*R*,9*S*-triols **7** and **8**, enantiomeric with **1** and **6**, respectively, were obtained from epoxyketol **4**. For reference purposes, we also prepared the two C-9 epimers of

*For part 65, see Ref. 1.

§For nomenclature, see Ref. 16.



7*E*-megastigmene-5*S*,6*S*,9-triol (**9**, **10**) and the corresponding enantiomers **11** and **12** from epoxyketols **3** and **5**, respectively. The spectral data of **9** (and **11**) and **10** (and **12**) agreed well with those previously reported for the racemic C-9 epimers of *trans*-5,6-dihydroxy-7*E*-megastigmen-9-ol.[6]

There are a few reports on the occurrence of 5,6-dihydroxy-7-megastigmenes in nature; e.g. extensively racemized *trans*-5,6-dihydroxy-7*E*-megastigmen-9-one has been isolated from tea,[7] 5*R*,6*R*-dihydroxy-7*E*-megastigmen-9-one (**13**) from *Rehmannia glutinosa var. purpurea*, and a corresponding D-glucoside (**14**) from *Aeginetia indica var. gracilis*.[8] While a plausible route to these *trans*-5,6-diols may involve acid-induced hydrolysis of 5*S*,6*R*-epoxy-7*E*-megastigmen-9-one (**15**),[9,10] the new tobacco constituent (**1**), having a *cis*-5,6-dihydroxy system, must arise via a different biogenetic pathway. Since a carotenoid precursor having an adequate end group has not as yet been found in tobacco, this pathway is sug-

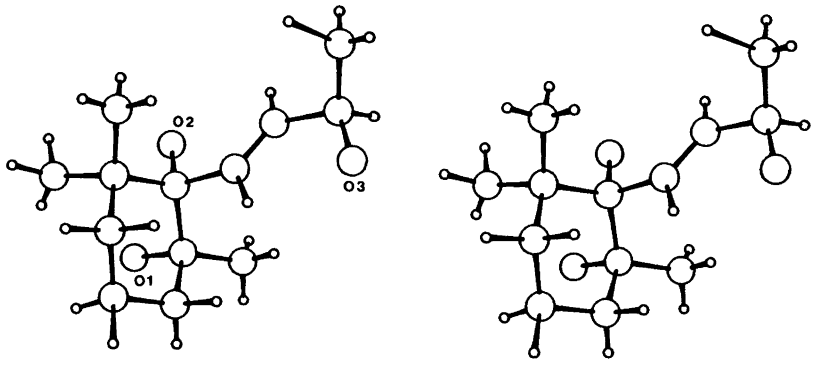
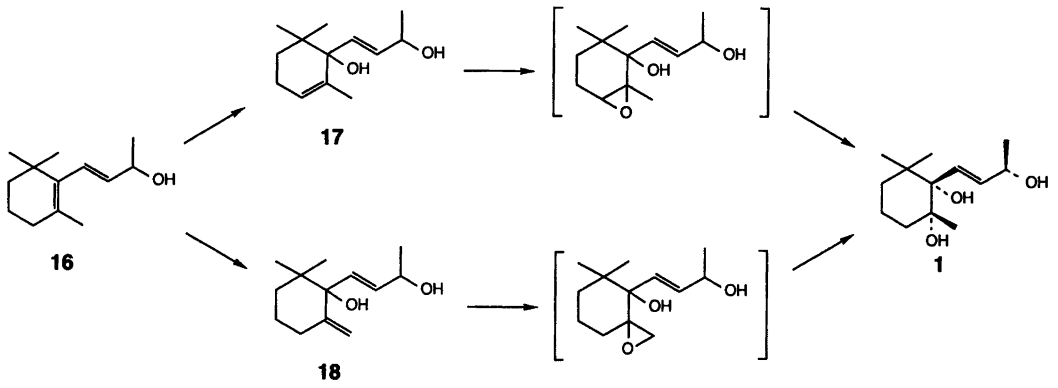


Fig. 1. A stereoscopic view of (5*R*,6*S*,7*E*,9*R*)-7-megastigmene-5,6,9-triol (**6**).



Scheme 1. Proposed biogenesis of *1*.

gested to have 5,7*E*-megastigmadien-9-ol (**16**) as a precursor and involve hydroxylation of 4,7*E*-megastigmadiene-6,9-diol (**17**) or 5(13), 7*E*-megastigmadiene-6,9-diol (**18**), possibly via reductive opening of the corresponding 4,5- or 5,13-epoxides. Support for the validity of this pathway is provided by the fact that **18**[11] is present in tobacco (Scheme 1).

In contrast to 3,6-epoxy-7*E*-megastigmene-5,9-diol (**19**), which is a tobacco constituent[12] having a 9*R*-configuration,[13] the new triol (**1**) has *S* chirality at C-9, implying the presence of different reductive enzymes in tobacco.

Experimental

With the exception of optical rotations, which were recorded on a Perkin-Elmer 241 polarimeter, the instruments specified in Ref. 14 were used.

Isolation. (5*R*,6*S*,7*E*,9*S*)-7-Megastigmene-5,6,9-triol (**1**, 1.3 mg) was isolated from fraction A3 of an extract obtained from 295 kg of sun-cured Greek tobacco (Serres)[15] by column chromatography on silica gel (hexane/EtOAc gradient) followed by HPLC using columns packed with μ-Bondapac/C_{18} (methanol/water 40:60) and μ-porasil (hexane/EtOAc 20:80). Compound **1** had m.p. 118.5–119.0 °C; $[\alpha]_D$ −5.0° (*c* 0.12, $CHCl_3$); [Found: $(M-18)^{+\cdot}$ 210.1616. Calc. for $C_{13}H_{22}O_2$: 210.1620]; IR ($CHCl_3$): 3611 and 3408 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (s) / 1.06 (s) (H−11 / H−12), 1.22 (s, H−13), 1.30 (d, *J* = 6.4 Hz, H−10), 4.39 (ddq, *J* = 0.8, 4.6 and 6.4 Hz, H−9), 5.85 (dd, *J* = 4.6 and 15.7 Hz, H−8), and 5.89 (d, *J* = 15.7 Hz, H−7); ^{13}C NMR ($CDCl_3$): δ 38.2 (C−1), 36.5 / 36.9 (C−2 / C−4), 18.6 (C−3), 75.0 / 79.1 (C−5 / C−6), 130.5 (C−7), 134.3 (C−8), 68.8 (C−9), 23.9 / 25.3 / 26.6 / 26.7 (C−10 / C−11 / C−12 / C−13); MS [*m/z* (%, composition)]: 210 (3, M−18), 192 (2, $C_{13}H_{20}O$), 177 (2, $C_{12}H_{17}O$), 165 (2, $C_{11}H_{17}O$), 149 (34, $C_{11}H_{17}$) 125 (21, $C_8H_{13}O$ and $C_7H_9O_2$), 109 (28, C_8H_{13} and C_7H_9O), 93 (15, C_7H_9), 83 (17), 69 (32, C_5H_9 and C_4H_5O), 55 (23) and 43 (100).

Reduction of (4R,5R,6S,7E)-4,5-epoxy-6-hydroxy-7-megastigmen-9-one (**2**). A solution of 16 mg of **2** in 5 ml of Et_2O was refluxed with an excess of LAH for 6h. Work-up and separation by HPLC (Spherisorb 5; hexane/EtOAc 20:80) yielded 3.9 mg of (5*R*,6*S*,7*E*,9*R*)-7-megastigmene-5,6,9-triol (**6**) and 4.3 mg of (5*R*,6*S*,7*E*,9*S*)-7-megastigmene-5,6,9-triol (**1**). The latter had m.p. 115.0–115.5 °C; $[\alpha]_D$ −2.4° (*c* 0.17, $CHCl_3$); the IR, mass, ^1H and ^{13}C NMR spectra were identical to those of the naturally occurring **1**. Triol **6** had m.p. 80.5–82.0 °C; $[\alpha]_D$ −6.3 °C (*c* 0.16, $CHCl_3$); IR ($CHCl_3$): 3609 and 3560 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (s) / 1.09 (s) (H−11 / H−12), 1.17 (s, H−13), 1.30 (d, *J* = 6.3 Hz, H−10), 4.39 (m, H−9), 5.87 (dd, *J* = 4.0 and 15.8 Hz) and 5.90 (d, *J* = 15.8 Hz, H−7); ^{13}C NMR ($CDCl_3$): δ 38.2 (C−1), 36.6 / 37.2 (C−2 / C−4), 18.4 (C−3), 74.9 / 79.1 (C−5 / C−6), 130.0 (C−7), 134.0 (C−8), 68.5 (C−9), 23.7 / 25.0 / 26.9 / 27.0 (C−10 / C−11 / C−12 / C−13); MS [*m/z* (%)]: 210 (1, M−18), 192 (1), 177 (2),

163 (3), 149 (26), 125 (16), 109 (31), 93 (11), 83 (19), 69 (40), 55 (28) and 43 (100).

Reduction of (4S,5S,6R,7E)-4,5-epoxy-6-hydroxy-7-megastigmene-9-one (**4**). By using conditions similar to those described above, **4** (41 mg) was converted to 10.4 mg of (*5S,6R,7E,9S*)-7-megastigmene-5,6,9-triol (**8**) and 12.6 mg of the corresponding 9*R*-epimer (**7**). Triol **8** had m.p. 78.0–81.0 °C and $[\alpha]_D$ +5.3° (*c* 0.62, $CHCl_3$), and triol **7** had m.p. 115.5–116.0 °C and $[\alpha]_D$ +1.2° (*c* 0.76, $CHCl_3$); their IR, ^1H NMR and mass spectra were identical with those of **6** and **1**, respectively.

Reduction of (4S,5S,6S,7E)-4,5-epoxy-6-hydroxy-7-megastigmen-9-one (**3**). By using conditions similar to those described above, **3** (9.5 mg) was converted to 3.0 mg of (*5S,6S,7E,9*ξ_1)-7-megastigmene-5,6,9-triol (**9**) and 1.7 mg of the corresponding 9-epimer **10**. Triol **9** had m.p. 111.0–112.0 °C; $[\alpha]_D$ +35° (*c* 0.11, $CHCl_3$); IR ($CHCl_3$): 3609, 3437 and 1602 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 38.0 (C–1), 36.3 (C–2 and C–4), 17.9 (C–3), 75.0 / 78.6 (C–5 / C–6), 130.3 (C–7), 134.7 (C–8), 68.7 (C–9), 23.9 / 25.0 / 26.4 / 26.9 (C–10 / C–11 / C–12 / C–13); the ^1H NMR and mass spectral data agreed well with those previously published for the isomer of (±)-7-megastigmene-5,6,9-triol having m.p. 86–87 °C.[6] Triol **10** was obtained as an oil, which had $[\alpha]_D$ +26° (*c* 0.14, $CHCl_3$); IR ($CHCl_3$): 3610, 3429 and 1603 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 38.0 (C–1), 36.3 (C–2 and C–4), 17.9 (C–3), 75.0 / 78.6 (C–5 / C–6), 130.5 (C–7), 134.7 (C–8), 68.9 (C–9), 24.0 / 25.0 / 26.3 / 26.9 (C–10 / C–11 / C–12 / C–13); the ^1H NMR and mass spectral data agreed well with those published for the isomer of (±)-7-megastigmene-5,6,9-triol having m.p. 112 °C.[6]

Reduction of (4R,5R,6R,7E)-4,5-epoxy-6-hydroxy-7-megastigmen-9-one (**5**). By using conditions similar to those described above, **5** (14.8 mg) was converted to 4.2 mg of (*5R,6R,7E,9*ξ_1)-7-megastigmene-5,6,9-triol (**11**) and 2.9 mg of the corresponding 9-epimer (**12**). Triol **11** had m.p. 111.0–111.5 °C; $[\alpha]_D$ −31° (*c* 0.34, $CHCl_3$); the IR, ^1H NMR and mass spectra were identical with those of **9**. Triol **12** was obtained as an oil, which had $[\alpha]_D$ −25° (*c* 0.15, $CHCl_3$); the IR, ^1H NMR and mass spectra were identical with those of **10**.

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