Tobacco Chemistry. 66.* (5R,6S,7E,9S)-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 (5R,6S,7E,9S)-7-megastigmene-5,6,9-triol by spectral methods, X-ray analysis of the corresponding 9R-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 C13-compounds clearly derived via oxidative cleavage of the polynene chain of cyclic carotenoids. As an addition to these we now report the isolation, structure determination and asymmetric synthesis of a new C13-triol.

**Results**

The new compound (1, C13H22O3, 1.3 mg) was isolated from sun-cured leaves of Greek tobacco. It contains a secondary hydroxy group [1H NMR signal at δ 4.39 (ddq); 13C NMR signal at δ 86.8 (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 [13C NMR signals at δ 75.0 (s) and 79.1 (s)]. Since the 1H 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 7E-megastigmene-5,6,9-triol.1

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., who have reported

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*For part 65, see Ref. 1.

†For nomenclature, see Ref. 16.


the asymmetric syntheses of the (4R,5R,6S,7E)- and (4S,5S,6S,7E)-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 5R,6S 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 P41. 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. A stereoscopic view, which summarizes the X-ray results and demonstrates that 6 is (5R, 6S,7E,9R)-7-megastigmene-5,6,9-triol, is shown in Fig. 1. As a result, triol 1 is assigned a 9S-configuration.

The 5S,6R,9R- and 5S,6R,9S-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
7E-megastigmene-5S,6S,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-7E-megastigmene-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-7E-megastigmene-9-one has been isolated from tea,7 5R,6R-dihydroxy-7E-megastigmen-9-one (13) from Rehmannia glutinosa var. purpurea, and a corresponding d-glucoside (14) from Aegi-netia indica var. gracilis.8 While a plausible route to these trans-5,6-diols may involve acid-induced hydrolysis of 5S,6R-epoxy-7E-megastigmene-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 (5R,6S,7E,9R)-7-megastigmene-5,6,9-triol (6).
gusted to have 5,7E-megastigmadien-9-ol (16) as a precursor and involve hydroxylation of 4,7E-megastigmadiene-6,9-diol (17) or 5(13), 7E-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 was present in tobacco (Scheme 1).

In contrast to 3,6-epoxy-7E-megastigmen-5,9-diol (19), which is a tobacco constituent having a 9R-configuration, 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.** (5R,6S,7E,9S)-7-Megastigmen-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) by column chromatography on silica gel (hexane/EtOAc gradient) followed by HPLC using columns packed with μ-Bondapac/C$_m$ (methanol/water 40:60) and μ-porasil (hexane/EtOAc 20:80). Compound I had m.p. 118.5–119.0 °C; [α]$_D$ $-5.0^\circ$ (c 0.12, CHCl$_3$); [Found: (M–18)$^+$ 210.1616. Calc. for C$_{13}$H$_{22}$O$_2$: 210.1620]; IR (CHCl$_3$): 3611 and 3408 cm$^{-1}$; $^1$H 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 (%)]: 210 (1, M–18), 192 (2, C$_{13}$H$_{20}$O), 177 (2, C$_{12}$H$_{17}$O), 165 (2, C$_{11}$H$_{15}$O), 149 (34, C$_{11}$H$_{15}$O), 125 (21, C$_{9}$H$_{13}$O and C$_{9}$H$_{13}$O), 109 (28, C$_{9}$H$_{13}$ and C$_{9}$H$_{13}$O), 93 (15, C$_{7}$H$_{9}$), 83 (17), 69 (32, C$_{7}$H$_{9}$ and C$_{7}$H$_{9}$O), 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$_2$O 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 (5R,6S,7E,9R)-7-megastigmen-5,6,9-triol (6) and 4.3 mg of (5R,6S,7E,9S)-7-megastigmen-5,6,9-triol (1). The latter had m.p. 115.0–115.5 °C; [α]$_D$ $-2.4^\circ$ (c 0.17, CHCl$_3$); the IR, mass, $^1$H and $^{13}$C NMR spectra were identical to those of the naturally occurring 1. Triol 6 had m.p. 80.5–82.0 °C; [α]$_D$ $-6.3^\circ$ (c 0.16, CHCl$_3$); IR (CHCl$_3$): 3609 and 3560 cm$^{-1}$; $^1$H 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), 457.
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-megastigmen-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-megastigmen-5,6,9-triol (8) and 12.6 mg of the corresponding 9R-epimer (7). Triol 8 had m.p. 78.0–81.0°C and [α]D +5.3° (c 0.62, CHCl₃), and triol 7 had m.p. 115.5–116.0°C and [α]D +1.2° (c 0.76, CHCl₃); 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,9S)-7-megastigmen-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; [α]D +35° (c 0.11, CHCl₃); IR (CHCl₃): 3609, 3437 and 1602 cm⁻¹; ¹C NMR (CDCl₃): 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-megastigmen-5,6,9-triol having m.p. 86–87°C.⁶ Triol 10 was obtained as an oil, which had [α]D +26° (c 0.14, CHCl₃); IR (CHCl₃): 3610, 3429 and 1603 cm⁻¹; ¹C NMR (CDCl₃): 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 / 26.5 / 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-megastigmen-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,9S)-7-megastigmen-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; [α]D −31° (c 0.34, CHCl₃); the IR, ¹H NMR and mass spectra were identical with those of 9. Triol 12 was obtained as an oil, which had [α]D −25° (c 0.15, CHCl₃); the IR, ¹H NMR and mass spectra were identical with those of 10.

Acknowledgements. We are grateful to Dr. Toshiaki Nishida for recording the NMR spectra, to Mr. Jacek Bielawski and Dr. Olof Dahlman for recording the mass spectra, and to Professor Peder Kierkegaard for his stimulating interest in the X-ray work. We are also indebted to Professor Conrad Hans Eugster for a generous gift of racemic trans-5,6-dihydroxy-7E-megastigmen-9-one.

References


Received April 9, 1987.