Acta Chemica Scandinavica B 31 (1977) 609—613

Tobacco Chemistry. 41. Structure Determination and Synthesis of 5(13), 7*E*-Megastigmadien-6,9-diol, a New Constituent of Greek Tobacco

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

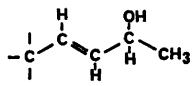
Research Department, Swedish Tobacco Co.,
P.O. Box 17007, S-104 62 Stockholm, Sweden

A new C_{13} nor-carotenoid was isolated from Greek *Nicotiana tabacum* L. and shown by spectroscopic methods and synthesis to be 5(13),7*E*-megastigmadien-6,9-diol.* The additional compounds obtained in the reaction of singlet oxygen with (±)-β-ionol were also characterized.

A large number of C_{13} compounds obviously formed by initial oxidative cleavage of carotenoids and subsequent chemical alterations have been isolated from tobacco.[2] The present paper deals with the structure elucidation and biomimetic synthesis of a new representative of this group of compounds. An account of the additional products obtained in the reaction of singlet oxygen with β-ionol is also given.

RESULTS

The new tobacco constituent, *1*, $C_{13}H_{22}O_2$, was isolated in minute quantity by repeated liquid chromatography of a polar, volatile fraction, B 8,[3] obtained from an ether extract of sun-cured Greek tobacco. It contained a secondary hydroxyl group as shown by IR absorption at 3600 and 3550 – 3120 cm^{-1} and a one-proton quintet at δ 4.39 in the ^1H NMR spectrum. This quintet was converted to a quartet on irradiation at the frequency of an olefinic doublet of doublets at δ 5.85 and to a doublet on irradiation at the frequency of a methyl doublet at δ 1.30. Since, conversely, irradiation at the frequency of the quintet at δ 4.39 converted the doublet of doublets at δ 5.85 to the A part of an AB system, δ_B 6.14, $J_{AB} = 15.5$ Hz, it follows that the new tobacco isolate incorporates partial structure A.



A

Moreover, it was clear from the presence of two three-proton singlets at δ 0.91 and one-proton signals at δ 4.86 and 4.91 in the ^1H NMR spectrum that *1* contains two methyl groups on fully substituted carbon(s) and an exocyclic methylene group (IR band at 1640 cm^{-1}). In view of these results and the elemental composition the nor-carotenoid structure *1* appeared highly probable.

Since the minute quantity available did not allow corroborative ^{13}C NMR studies, the proposed structure of *1* was confirmed by synthesis. This was achieved by subjecting (±)-β-ionol (*2*) to photooxygenation sensitized by rose bengal followed by treatment of the reaction mixture with Na_2SO_3. The main product thus obtained, 5(13),7*E*-megastigmadien-6,9-diol, whose ^{13}C NMR spectrum contained diagnostically important peaks at δ 151.31 (s), 108.86 (t) and at 79.25/79.17 (s) due to the $>C=CH_2$ and $-\overset{|}{C}-OH$ groups, respectively (*cf.* Table 1), gave IR, ^1H NMR and mass spectra identical

* For nomenclature: see Ref. 1.

with those of the new tobacco constituent (*1*). This synthetic product (*1*) is evidently formed in an ene-reaction involving an attack of singlet oxygen on the tetra-substituted 5,6 double bond and loss of a hydrogen from C-13 in (±)-β-ionol (*2*). This attack should occur at either side of the molecule and should furnish two pairs of diastereoisomers, a view corroborated by the fact that the signals ascribed to C-4, and C-6 to C-10 are doublets in the proton noise-decoupled ^{13}C NMR spectrum of the synthetic sample. The stereochemistry and absolute configuration of the natural product (*1*) could not be determined due to shortage of material.

In addition to the major product (*1*) from the reaction of singlet oxygen with (±)-β-ionol (*2*), a few further compounds were isolated and identified. Of these, 4,7-megastigmadien-6,9-diol (*3*) and 6,7-megastigmadien-5,9-diol (*4*) can be viewed as products of ene-reactions, whereas 5,8-epidioxy-6-megastigmen-9-ol (*5*), dihydroactinidiolide (*6*) and 5,6-7,8-diepoxymegastigman-9-ol (*7*) probably arise *via* a 1,4-addition reaction.

The presence of the 4,5 double bond in 4,7-megastigmadien-6,9-diol (*3*) was disclosed by the ^1H NMR spectrum, which contained a broad three-proton signal at δ 1.61 and a one-proton multiplet at δ 5.50 corresponding to a methyl group allylically coupled to an olefinic proton. The ^{13}C NMR spectrum, which in addition to the C-7 and C-8 sp^3 carbon signals displayed resonances due to two sp^2 carbon atoms, a doublet at δ 122.09 and a singlet at δ 136.91, was in accordance with the structure proposed for *3*. Moreover, the mass spectrum of *3* had a prominent peak at *m/e* 154 corresponding to a $C_9H_{14}O_2$ fragment, which arises by retro-Diels-Alder cleavage of the molecular ion induced by the 4,5 double bond (Fig. 1).

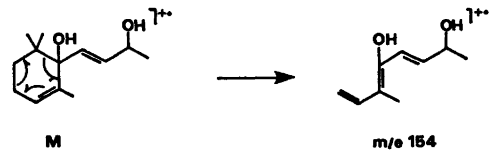
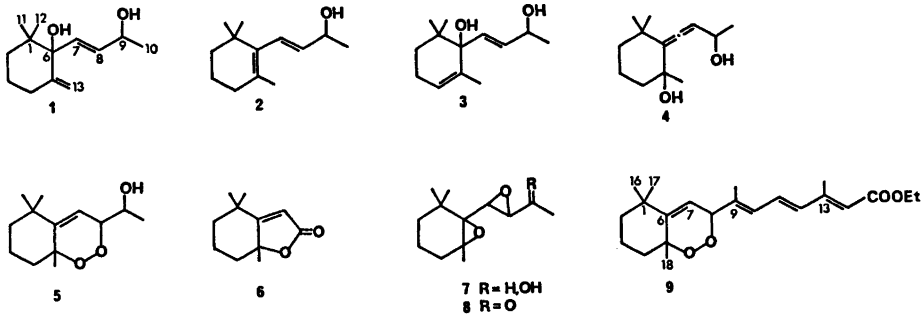


Fig. 1. The retro-Diels-Alder cleavage of the molecular ion.

6,7-Megastigmadien-5,9-diol (*4*) gave a ^{13}C NMR spectrum having signals at δ 117.47, 196.66 and 99.59 corresponding to the carbon atoms of the trisubstituted allenic group and an IR spectrum having a characteristic allene band at 1965 cm^{-1}.

5,8-Epidioxy-6-megastigmen-9-ol (*5*) was isolated as a 1:1 mixture with dihydroactinidiolide (*6*, *vide infra*). Its structure was deduced from the ^{13}C NMR spectrum of this mixture. Thus, subtraction of the signals due to dihydroactinidiolide (*6*) left thirteen signals to be assigned to *5*. Eleven of these had chemical shift values close to those previously published for the C-1 to C-8 and C-16 to C-18 signals for compound *9*.[4] Since the remaining two signals were due to an oxygen-bearing sp^3 methine carbon and a methyl carbon it was reasonable to formulate *5* as 5,8-epidioxy-6-megastigmen-9-ol. The mass spectrum having the base peak at *m/e* 181, which corresponds to an ion formed by a favoured rupture of the 8,9 bond in the molecular ion, provides supporting evidence for the structure proposed.

(±)-Dihydroactinidiolide (*6*) obtained in a pure state from a different fraction, was identified by spectral comparison with an authentic sample.



As shown by GC-MS analysis and by the ^{13}C and ^{1}H NMR spectra, 5,6-7,8-diepoxymegastigman-9-ol (*7*) was obtained as a mixture of isomers. The presence of the 7,8-epoxide group adjacent to the secondary hydroxyl group at C-9 was disclosed by the ^{1}H NMR spectrum of this mixture, which exhibited a one-proton doublet at δ 3.38/3.42 ($J = 2.1$ Hz), a one-proton doublet of doublets at δ 3.04/3.09 ($J =$ 2.1 and 3.3 Hz), a one-proton doublet of quartets at δ 3.76/3.97 ($J = 3.3$ and 6.5 Hz), and a three-proton doublet at δ 1.27/1.30 ($J = 6.5$ Hz). The ^{13}C NMR spectrum not only confirmed the presence of the 7,8-epoxide group, δ 57.26/56.96 (d) and 60.24/59.48 (d), but also suggested that *7* incorporated a tetra-substituted epoxide group, δ 66.10 (s) and 63.80/63.59 (s). This must be attached to C-5 and C-6, a conclusion supported by the fact that the ^{1}H NMR spectrum contained a methyl singlet at δ 1.31/1.36.

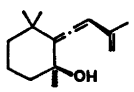
Oxidation using Jones' reagent converted the alcohol (*7*) to 5,6-7,8-diepoxymegastigman-9-one (*8*), whose IR spectrum had an absorption band at 1710 cm^{-1} and whose ^{1}H NMR spectrum contained a three proton singlet at δ 2.09 and an AB quartet centered at δ 3.47 corresponding to the protons of the methyl ketone and 7,8-epoxy groups, respectively. The facts that no doubling of peaks was observed in either the ^{1}H or the ^{13}C NMR spectrum of the ketone (*8*) and that the sample was optically inactive support the view that the mixture of alcohols (*7*) is derived from the two pairs of diastereomeric 5,8-epidioxy-6-megastigmen-9-ols (*5*).

Sensitized photooxygenation of β-ionol (*2*), performed in the presence of catalytic amounts of alkali, has previously been studied by Isoe *et al.*[5] They isolated dihydroactinidiolide (*6*) as the major product and identified one of the minor components as 6,7-megastigmadien-5,9-diol (*4*). The latter compound, which has been patented as a flavour additive to tobacco, and the 7*Z*-derivative of *1* are major products of the photooxidation of *cis*-β-ionol.[6] Compounds *10*–*15* have been obtained on photooxygenation of 3-methyl-1-(2,6,6-trimethylcyclohexen-1-yl)-1,3-butadiene (*16*),[7] a result which is analogous to our findings for the reaction of β-ionol (*2*) with singlet oxygen.

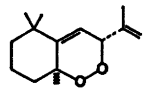
EXPERIMENTAL

IR spectra were measured on Digilab FTS-14 and Perkin-Elmer 257 instruments and mass spectra on an LKB 2091 instrument. Accurate mass measurements were carried out on an Atlas SM 1 instrument at the Laboratory for Mass Spectrometry, the Karolinska Institute, Stockholm. Fourier transform ^{1}H NMR (100 MHz) and ^{13}C NMR (25.16 MHz) spectra were obtained on a Varian XL-100-12 spectrometer equipped with 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 × 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.

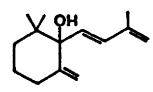
Isolation of 5(13), 7E-megastigmadien-6,9-diol (1) from tobacco. A volatile, neutral fraction (B8)[3] of an extract obtained from 295 kg of sun-cured Greek *Nicotiana tabacum* L. was chromatographed over silica gel using a light petrol/diethyl ether gradient. One of the subfractions obtained was purified further by liquid chromatography using columns packed with Bondapak C_{18}/Porasil (Waters), μ-Bondapak C_{18} (Waters) and μ-Porasil (Waters) to afford 3 mg of *1* as a colourless oil (Found: M 210.1620. Calc. for $C_{13}H_{22}O_2$: 210.1620), which had IR ($CHCl_3$) bands at 3600 (m), 3550–3120 (w) and 1640 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ



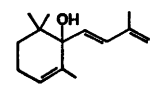
10



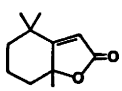
11



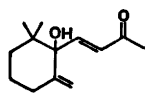
12



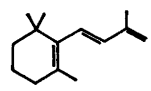
13



14



15


16

0.91 (6 H, s), 0.93 (1 H, s), 1.30 (3 H, d, $J=6$ Hz), 4.39 (1 H, quintet, $J=6$ Hz), 4.86 (1 H, m), 4.91 (1 H, m), 5.85 (1 H, dd, $J=15.5$ and 6 Hz) and 6.14 (1 H, broad d, $J=15.5$ Hz) (AB part of an ABX system); MS [*m/e* (composition, %)]: 210 ($C_{13}H_{22}O_2$, 2), 192 ($C_{13}H_{20}O$, 4), 177 ($C_{12}H_{17}O$, 3), 165 ($C_{11}H_{17}O$, 5), 152 ($C_{10}H_{16}O$, 68), 141 ($C_8H_{13}O_2$, 30), 123 (C_9H_{15} and $C_8H_{11}O$, 22), 109 (C_8H_{13} and C_7H_9O, 42), 97 (C_7H_{13} and C_6H_9O, 38), 96 (C_7H_{12} and C_6H_8O, 27), 95 (C_7H_{11} and C_6H_7O, 43), 81 (29), 69 (33), 55 (28) and 43 (100).

Photooxidation of β-ionol. A solution of 1.0 g of (±)-β-ionol and 0.1 g of rose bengal in 25 ml of methanol in a tube cooled by a water jacket was irradiated for 1 h with a 400 W sodium high pressure lamp placed outside the tube while oxygen was bubbled through the reaction mixture. A solution of 1.0 g of Na_2SO_3 in 20 ml of H_2O was added and the reaction mixture was stirred at room temperature for 17 h. Dilution with water, extraction with ether and chromatography on silica gel using a hexane/ethyl acetate gradient eluted in order compounds *5*, *6*, *7*, *1*, *3*, and *4*. Further purification was achieved by high performance liquid chromatography on columns packed with μ-Porasil (Waters) using hexane and ethyl acetate as eluent.

5(13),7E-megastigmadien-6,9-diol (*1*, 134 mg) gave IR, ^{1}H NMR and mass spectra identical to those of the natural product.

4,7-Megastigmadien-6,9-diol (*3*, 9 mg), which was recrystallized from diethyl ether–hexane (1:1) to give white needles, m.p. 108–109 °C. (Found: $[M-H_2O]^+$ 192.1518. Calc. for $C_{13}H_{20}O$: 192.1514). IR bands at 3600 (s), and 3550–3100 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.90 (3 H, s), 0.98 (3 H, s), 1.30 (3 H, d, $J=6$ Hz), 1.61 (3 H, m), 4.39 (1 H, m, $W_{1/2}=14$ Hz), 5.50 (1 H, m, $W_{1/2}=10$ Hz) and 5.6–5.8 (2 H, overlapping signals); MS [*m/e* (composition, %)]: 192 (M–18, $C_{13}H_{20}O$, 17), 154 ($C_9H_{14}O_2$, 68), 136 ($C_9H_{12}O$, 43), 121 (C_9H_{13}, 37), 111 ($C_7H_{11}O$, 100), 109 (C_8H_{13} and C_7H_9O, 63), 96 (C_6H_8O, 44), 93 (C_7H_9, 48), 83 (C_6H_{11} and C_5H_7O 56), 55 (38) and 43 (88).

6,7-Megastigmadien-5,9-diol (*4*, 5 mg), which was recrystallized from ethyl acetate to give white needles, m.p. 140–143 °C. (Found: $[M-H_2O]^+$ 192.1512. Calc. for $C_{13}H_{20}O$: 192.1514). IR (KBr) bands at 3650–3040 (s) and 1965 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.06 (3 H, s), 1.26 (3 H, s), 1.32 (3 H, d, $J=6$ Hz), 1.36 (3 H, s), 4.76 (1 H, quintet, $J=6$ Hz) and 5.45 (1 H, d, $J=6$ Hz); MS [*m/e* (composition, %)]: 210 (M, 1), 192 ($C_{13}H_{20}O$, 10), 177 ($C_{12}H_{17}O$, 28), 149 ($C_{11}H_{17}$ and $C_{10}H_{13}O$, 17), 133 ($C_{10}H_{13}$, 30), 119 (C_9H_{11}, 23), 107 (C_8H_{11} and C_7H_7O, 100), 85 (24) and 43 (88). Acetylation of *4* with acetic anhydride and pyridine at room temperature for 30 min afforded *9-acetoxy-6,7-megastigmadien-5-ol* as a colourless oil, which had IR ($CHCl_3$) bands at 3580 (m), 3560–3300 (m), 1955 (m) and

Table 1. Carbon-13 chemical shifts and assignments for compounds *1*, *3*–*8*.[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11/C-12	C-13
1	39.14	37.24	22.66	33.04	151.31	79.25	131.39	134.21	68.59	23.64	24.16/22.88	108.86
				32.98		79.17	131.27	133.97	68.39	23.54		
3[b]	36.61	33.10	22.47	122.09	131.91	76.76	131.33	134.07	66.61	22.38	24.27/24.52	18.65
4[b]	33.29	40.26[c]	17.96	40.48[c]	68.73	117.47	196.66	99.59	64.08	23.88	31.87[d]/30.83[d]	28.90[d]
5	35.13	40.73	18.75	35.65	79.57	149.00	115.01	82.67	70.21	19.72	25.49[e]/30.48[c]	27.77[c]
6	36.32	40.07	19.53	41.52	86.80	182.26[e]	112.18	172.23[e]			24.05/29.70	24.30
7	33.41	38.43	17.10	32.02	63.80	66.10	57.26	60.24	66.04	20.01	25.27/26.79	20.01
	33.36	38.37		31.90	63.59		56.96	59.48	65.82	19.40		
8	33.39	38.17	17.03	31.80	63.92	65.66	58.94[c]	57.92[c]	*f*	24.86	25.02/26.78	19.69

[a] δ-Values relative to TMS; spectrum recorded in $CDCl_3$. [b] Spectrum recorded in DMSO-d_6. [c,d] Assignment may be reversed. [e] The distinction between the C-6 and C-8 signals was made with the aid of the proton-coupled ^{13}C NMR spectrum; the sharp doublet ($^2J_{CH}=8.3$ Hz) at δ 171.23 was assigned to C-8 and the signal at δ 182.26, broadened due to several three bond C-H couplings, was assigned to C-6. [f] Not visible due to low sample concentration.

1725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.04/1.05 (3 H, s), 1.22/1.24 (3 H, s), 1.32/1.34 (3 H, s), 1.37 (3 H, d, $J = 6$ Hz), 2.04/2.05 (3 H, s) and 5.15–5.55 (2 H, overlapping signals). These data are somewhat at variance with previously published values.[5]

5,8-Epidioxy-6-megastigmen-9-ol (*5*, 7 mg), was present as a 1:1 mixture with dihydroactinidiolide (*6*). Subtraction of the peaks due to *6* from the ^1H NMR spectrum ($CDCl_3$) of the mixture left the following signals for *5*: δ 1.12 (3 H, s), 1.17 (3 H, s), 1.26 (3 H, d, $J = 6$ Hz), 1.61 (3 H, s), 4.15 (2 H, overlapping signals) and 5.67 (1 H, d, $J = 3$ Hz); MS [*m/e* (%)]: 208 (M−18, 1), 193 (2), 181 (100), 163 (13), 135 (22), 121 (18), 107 (18), 95 (18), 81 (11), 69 (17), 55 (13) and 43 (58).

(±)-Dihydroactinidiolide (*6*, 103 mg) obtained in a pure form from a more polar fraction, was identified by comparison of its IR, ^1H NMR and mass spectra with those of an authentic sample.

5,6-7,8-Diepoxymegastigman-9-ol (*7*, 25 mg), which was recrystallized from hexane to give white needles, m.p. 91–93 °C. (Found: $[M-45]^+$ 181.1221. Calc. for $C_{11}H_{17}O_2$: 181.1228). ^1H NMR ($CDCl_3$): δ 1.09 (3 H, s), 1.27/1.30 (3 H, d, $J = 6.5$ Hz), 1.28 (3 H, s), 1.31/1.36 (3 H, s), 3.04/3.09 (1 H, dd, $J = 3.3$ and 2.1 Hz), 3.38/3.42 (1 H, d, $J = 2.1$ Hz), 3.76/3.97 (1 H, dq, $J = 6.5$ and 3.3 Hz); MS [*m/e* (composition, %)]: 208 (M−18, 1), 193 (1), 181 ($C_{11}H_{17}O_2$, 13), 163 ($C_{11}H_{15}O$, 10), 145 ($C_{11}H_{13}$, 6), 123 (C_8H_{15} and $C_8H_{11}O$, 39), 121 (C_9H_{13} and C_8H_9O, 22), 109 (C_8H_{13} and C_7H_9O, 17), 105 (C_8H_9, 15), 95 (C_7H_{11} and C_6H_7O, 22), 85 (C_5H_9O and $C_4H_5O_2$, 29), 71 (15), 69 (18), 67 (12), 55 (17) and 43 (100). Treatment of *7* with Jones' reagent for 1 h at room temperature afforded *5,6-7,8-diepoxymegastigman-9-one* (*8*) which had an IR band at 1710 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.07 (3 H, s), 1.30 (3 H, s), 1.31 (3 H, s), 2.09 (3 H, s), 3.43 (1 H, d, $J = 2$ Hz) and 3.51 (1 H, d, $J = 2$ Hz); MS [*m/e* (%)]: 206 (M−18, 1), 181 (12), 163 (10), 145 (3), 137 (6), 123 (29), 121 (17), 105 (15), 95 (21), 85 (27), 69 (16), 55 (17) and 43 (100).

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

REFERENCES

1. Aasen, A. J., Kimland, B., Almqvist, S.-O. and Enzell, C. R. *Acta Chem. Scand. 26* (1972) 2573.
2. Enzell, C. R., Wahlberg, I. and Aasen, A. J. *Fortschr. Chem. Org. Naturst. In press.*
3. Kimland, B., Aasen, A. J. and Enzell, C. R. *Acta Chem. Scand. 26* (1972) 2177.
4. Englert, G. *Helv. Chim. Acta 58* (1975) 2367.
5. Isoe, S., Hyeon, S. B., Ichikawa, H., Katsumura, S. and Sakan, T. *Tetrahedron Lett.* (1968) 5561.
6. Isoe, S., Hayase, Y., Kim, T., Okamoto, M., Funatsuki, G. and Sakan, T. *Japan. Kokai* (1976) 76 48,633; *Chem. Abstr. 85* (1976) 176948j.
7. Foote, C. S. and Brenner, M. *Tetrahedron Lett.* (1968) 6041.

Received April 4, 1977.