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Tobacco Chemistry. 24. (9*R*)-9-Hydroxy-4-megastigmen-3-one, a New Tobacco Constituent

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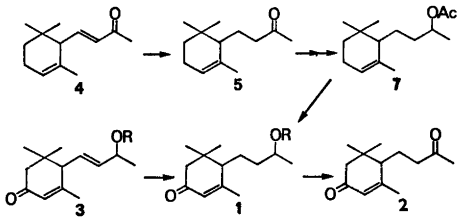
(9*R*)-9-Hydroxy-4-megastigmen-3-one has been isolated from Greek tobacco. Its absolute configuration was determined by correlation with derivatives of (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one.

Some time ago we reported on the presence in Greek tobacco, *Nicotiana tabacum* L., of (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one** (*8*, R=H, 3-oxo-α-ionol),[1] the absolute configuration of which has recently been established.[2] In the present communication we discuss the absolute configuration and synthesis of a closely related compound not previously encountered in tobacco.

The new compound, $C_{13}H_{22}O_2$, was isolated as its acetate from a complex, medium volatile fraction [3] of an extract [4] of Greek tobacco. The presence of a secondary hydroxyl group was apparent from an NMR deacylation shift [5] (1.1 ppm) observed on hydrolysis, and the formation of a methyl ketone when subjecting the resulting alcohol to oxidation. The second oxygen atom is part of a β,β-disubstituted, conjugated ketone grouping judging from its UV (237 nm), IR (1667 cm^{-1}) and NMR (one-proton multiplet at δ 5.84) spectra. Spin-spin decoupling experiments revealed that the single olefinic proton is coupled to a vinylic methyl group (δ 1.99, *J* 1.4 Hz) thereby unveiling one of the β-substituents. An AB-system at δ 2.02 and 2.36 (*J* 17 Hz) ascribed to a methylene group flanked by a carbonyl group and a fully substituted carbon atom, revealed the partial structure $-C\text{-}CH_2\text{-}CO\text{-}CH=C(CH_3)-$. The above evidence, and the fact that the NMR spectrum also disclosed the presence of two non-coupled methyl groups (singlets at δ 1.03 and 1.06) made structure *1* (R=H) appear likely for this new tobacco constituent.***

Its gross structure was confirmed synthetically by comparison of the acetate *9* (R=Ac) with corresponding racemic material obtained both on selective hydrogenation of (±)-9-acetoxy-4,7*E*-megastigmadien-3-one [2] (*3*, R=Ac), and on selective hydrogenation of (±)-*trans*-α-ionone (*4*), followed by hydride reduction, acetylation, and allylic oxidation.



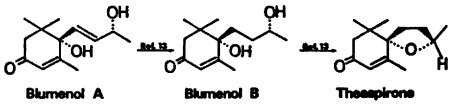
Galbraith and Horn [6] have recently isolated from *Podocarpus blumei* Endl. three structurally related compounds designated blumenol A, B, and C, the first one probably being identical to vomifoliol [9] (*Rauwolfia vomitoria*), and the last being identical to or stereoisomeric with the new tobacco compound. The structures of the blumenols were deduced from spectral data

** Nomenclature and stereochemistry as defined in Ref. 6.

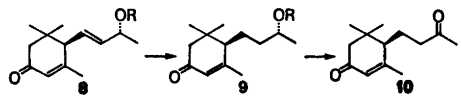
*** Dr. D. L. Roberts, R. J. Reynolds Tobacco Co., Winston-Salem, has simultaneously and independently identified *1* (R=H) as a new tobacco compound (private communication). Racemic *1* (R=H) has previously been reported as an intermediate in a synthesis of a tobacco additive, however without characterization.[7]

(molecular weight given for blumenol C, 208, is a misprint for 210; private communication), and in the cases of blumenol A and B, confirmed synthetically. Based on a comparison of the ORD curve of blumenol C with those of (+)-α-ionone [10] and lutein [11] the absolute configuration at C-6 was proposed as *R* which is consistent with the C-6(*S*) configurations of blumenols A and B very recently established chemically.[12,13]

Weiss *et al.*[13] also demonstrated chemically that the configuration at C-9 of blumenol A and B is *R* by converting (with stereospecific inversion at C-9) blumenol B into theaspirone of known absolute configuration (9*S*). However, contradictory to these results, Galbraith and Horn [12] have simultaneously suggested, on the basis of an assumed biogenetic relationship between the blumenols and theaspirone, that the absolute configuration of all the blumenols is *S* at C-9. It was therefore considered necessary to elucidate the absolute configurations of the new tobacco compound through correlation with synthetic material of known absolute stereochemistry.



The natural compound and its acetate displayed optical rotations somewhat higher than those of the corresponding material which could be prepared from (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one (*8*, R = H, *cf.* Table 1) and it was therefore imperative to examine the two chiral centres, C-6 and C-9, separately. The absolute configuration at C-6 was established as *R* by comparison of two samples of the diketone *10* derived on the one hand from the natural product *9* (R = H) and on the other from (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one (*8*, R = H), which both exhibited rotations of the same sign and similar magnitude (*cf.* Table 1). The somewhat lower optical activity of the latter material is presumably due to partial epimerization at C-6 during isolation which required basic conditions.



The absolute configuration at C-9 was disclosed by comparing the optical activities of the epimeric mixtures obtained after subjecting the natural alcohol *9* (R = H) and the correponding synthetic specimen obtained on hydrogenation of (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one (*8*, R = H) to alkali treatment. Depending on the configuration at C-9, the resulting equilibrium mixtures of epimers would comprise either the 6*R*,9*R* and 6*S*,9*R* or the 6*R*,9*S* and 6*S*,9*S* isomers. Since the components of one mixture would be the enantiomers of those in the other, the equilibrium constants have to be the same in both cases, and hence, the signs of the resulting rotations would reveal whether the configuration at C-9 is *R* or *S*. The two mixtures were found to exhibit optical activities of the same sign and similar magnitude thereby establishing the absolute configuration at C-9 of the new compound as *R*, which is the same as that found by Weiss *et al.*[13] for blumenol A and B. If, as assumed previously,[12] the blumenols are biogenetically interrelated it is considered likely that blumenol C has 9*R* configuration rather

Table 1. Optical activities, [α], of specimens of the natural product (9*R*)-9-hydroxy-4-megastigmen-3-one (*9*, R = H) and its derivatives, and of corresponding authentic compounds derived from (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one (*8*, R = H).

Natural [α](nm)	9 (R = H)	9 (R = Ac)	10	Authentic 9 (R = H)	9 (R = Ac)	10
365	+514°	+621°	+456°	+356°	+420°	+382°
436	+147.1°	+219°	+138°	+101.7°	+144.7°	+115°
546	+ 66.4°	+107	+ 64.6°	+ 46.4°	+ 72°	+ 53.6°
578	+ 55.9°	+ 92.2°	+ 56°	+ 39.4°	+ 61.8°	+ 45.5°
589	+ 54°	+ 87.5°	+ 52.6°	+ 37.2°	+ 57.8°	+ 44°
c($CHCl_3$)	0.22	0.31	0.34	0.33	0.55	0.44

than 9*S* as suggested by Galbraith and Horn.[12]

The structural relationship, including absolute configurations, between the two tobacco compounds (9*R*)-9-hydroxy-4-megastigmen-3-one (*9*, R=H) and (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one (*8*, R=H) indicates that they are biogenetically interrelated and likely to be derived from a common carotenoid precursor, *e.g.* lutein and/or α-carotene which are known tobacco carotenoids [14] possessing the same absolute configuration in the position corresponding to C-6.[10,11] The new compound *9* (R=H) might in turn be the precursor of two stereomers of 1,3,7,7-tetramethyl-2-oxa-bicyclo[4.4.0]-decan-9-one which were isolated recently from tobacco.[15] The conversion of *1* (R=H) to the latter compounds has been achieved chemically.[7]

EXPERIMENTAL

NMR, IR, UV, and mass spectra were recorded on Varian XL-100, Digilab FTS-14, Beckmann DK-2A, and LKB 9000 (70 eV) instruments, respectively. Optical activities were recorded on a Perkin-Elmer 141 polarimeter. Accurate mass determinations were carried out at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm.

Isolation. (9*R*)-9-Hydroxy-4-megastigmen-3-one (9, R=H) was isolated as its acetate (*9*, R=Ac, 25 mg) from a medium-volatile fraction of an extract from 295 kg Greek tobacco, *Nicotiana tabacum* L., using liquid chromatography. The sub-fractionation of this medium-volatile material will be described later.[3]

(9R)-9-Acetoxy-4-megastigmen-3-one (9, R=Ac). $[\alpha]_{20}$: see Table 1; λ_{max} (EtOH): 237 nm (ε 6940); ν_{max} (film): 2965 (s), 2940 (s), 2873 (m), 1737 (s), 1667 (s), 1376 (m), 1245 (s), 1134 (w), 1074 (w), 1023 (w), 949 (w), 896 (w), 844 (w), 671 (w), 611 (w) cm^{-1}; MS: *m/e* 252 (M^+, 30), 177 (26), 150 (35, 138 (36), 136 (33), 135 (50), 123 (24), 121 (29), 109 (29), 108 (62), 93 (42), 55 (29), 43 (100), 41 (45); accurate mass determination: $C_{15}H_{24}O_3$: Found: 252.1732, Calc.: 252.1725; δ ($CDCl_3$): 1.03 (3 H, s), 1.06 (3 H, s), 1.23 (3 H, d, *J* 6.3 Hz), 1.99 (3 H, d, *J* 1.4 Hz), 2.04 (3 H, s), 2.02 and 2.36 (2 H, AB-system, *J* 17 Hz), 4.86 (1 H, m), 5.84 (1 H, m); irradiation at δ 5.84 collapsed the doublet at δ 1.99 to a singlet.

(9R)-9-Hydroxy-4-megastigmen-3-one (9, R=H). The acetate (*9*, R=Ac, 12 mg) was dissolved in 1 % KOH/MeOH (6 ml) and left at room temperature for 2 h. The mixture was diluted with water and extracted with ether. Removal of the solvent left a colourless, TLC-pure oil (9 mg). $[\alpha]_{20}$: see Table 1; ν_{max} (film): 3420 (broad), 2967 (s), 2937 (s), 2872 (m), 1660 (s), 1419 (w), 1379 (m), 1325 (w), 1300 (m), 1258 (m), 1180 (w), 1122 (m), 1081 (w), 989 (w), 945 (w), 898 (w), 840 (w) cm^{-1}; MS: *m/e* 210 (M^+, 41), 150 (48), 135 (76), 123 (47), 109 (70), 108 (86), 95 (62), 93 (59), 69 (55), 55 (51), 45 (49), 43 (100), 41 (88); δ ($CDCl_3$): 1.02 (3 H, s), 1.07 (3 H, s), 1.20 (3 H, d, *J* 6.2 Hz), 2.0 (3 H, d, *J* 1.2 Hz), 2.01 and 2.40 (2 H, AB-system, *J* 17 Hz), 2.75 (OH), 3.76 (1 H, m), 5.83 (1 H, m).

4-Megastigmen-3,9-dione (10). The alcohol (*9*, R=H, 9 mg) was oxidised with CrO_3 to the diketone (*10*, 8 mg) employing the two-phase system described by Brown *et al.*[16] The conversion to the ketone was complete in 30 min, after which the aqueous phase was extracted with ether, washed with $NaHCO_3$, and concentrated leaving a colourless, TLC-pure oil. $[\alpha]^{20}$, see Table 1; ν_{max} (film): 2967 (s), 2938 (m), 2876 (m), 1718 (s), 1662 (s), 1441 (m), 1418 (m), 1379 (m), 1370 (m), 1291 (w), 1252 (m), 1180 (w), 1163 (w), 1121 (w), 1070 (w), 1024 (w), 971 (w), 953 (w), 869 (w), 838 (w) cm^{-1}; MS: *m/e* 208 (M^+, 40), 151 (73), 138 (25), 136 (71), 123 (31), 109 (67), 108 (31), 107 (21), 95 (40), 81 (23), 67 (23), 55 (23), 43 (100), 41 (40); δ ($CDCl_3$): 1.04 (3 H, s), 1.08 (3 H, s), 2.02 (3 H, d, *J* 1.1 Hz), 2.16 (3 H, s), *ca.* 2.28–2.57 (4 H, m), 5.85 (1 H, m), irradiation at δ 5.85 simplified the doublet at δ 2.02 to a singlet.

Preparation of (±)-9-acetoxy-4-megastigmen-3-one (1, R=Ac). (a): (±)-9-Acetoxy-4,7*E*-megastigmadien-3-one [3] (*3*, R=Ac, 60 mg) in dry dioxane (0.25 ml) was added to a suspension of Pd/C (10 %, 14 mg) in dioxane [17] (10 ml) which had been saturated with H_2. The theoretical amount of H_2 was consumed at atmospheric pressure in 40 min, after which the mixture was diluted with water, extracted with ether and concentrated. The colourless oil was chromatographed on silica gel furnishing pure acetate (*1*, R=Ac, 29 mg); its spectral data were identical to those of the acetate derived from the natural alcohol (*9*, R=H). (b): (±)-*trans*-α-Ionone (*4*, 12 g) dissolved in 0.25 N NaOH/EtOH (100 ml) was hydrogenated [18] at atmospheric pressure and room temperature using Pd/C (10 %, 250 mg) as catalyst. One equivalent of H_2 was taken up in 2.5 h after which water was added and the product (*5*, 12 g) extracted with pentane; δ ($CDCl_3$): 0.87 (3 H, s), 0.92 (3 H, s), 1.67 (3 H, d, *J* 1.0 Hz), 2.11 (3 H, s), 2.46 (2 H, t, *J* *ca.* 7 Hz), 5.33 (1 H, m). The dihydroionone (*5*, 1 g) was treated with $NaBH_4$ (300 mg) in EtOH (25 ml) for 1 h. The mixture was diluted with water and extracted with ether. Removal of the solvent left a colourless oil (6, 950 mg); δ ($CDCl_3$): 0.83 (3 H, s), 0.88 (3 H, s), 1.14 (3 H, d, *J* 6 Hz), 1.62 (3 H, d, *J* 1 Hz), *ca.* 1.9 (2 H, m), 2.6 (OH), 3.62 (1 H, m), 5.24 (1 H, m). The alcohol (*6*, 950 mg) was acetylated using acetic anhydride (1 g) in pyridine (10 ml). After 3 h at ambient temperature excess anhydride was destroyed with MeOH, diluted with aqueous sulphuric acid and extracted with ether. Removal of the solvent left the acetate (*7*, 980 mg) as a colourless oil:

δ ($CDCl_3$): 0.86 (3 H, s), 0.90 (3 H, s), 1.19 (3 H, d, J 6 Hz), 1.66 (3 H, d, J 1 Hz), 2.0 (3 H, s), 4.84 (1 H, m), 5.30 (1 H, m). The acetate (*7*, 950 mg) dissolved in acetic acid (5 ml) was added to a solution of CrO_3 (1 g) in acetic acid (15 ml) and stirred at room temperature for 3 h. Water was added and the mixture extracted with ether which was washed with $NaHCO_3$ and water. The residue obtained after distillation of the solvent was chromatogaphed on silica gel furnishing starting material (169 mg) and the ketoacetate (*1*, R=Ac, 177 mg), respectively. The spectral data of the product were indistinguishable from those of the acetate derived from the natural alcohol (9, R=H).

Preparation of (±)-9-hydroxy-4-megastigmen-3-one (1, R=H) and (±)-4-megastigmen-3,9-dione (2). The racemic acetate, prepared above, was hydrolyzed to the corresponding alcohol (*1*, R=H) which subsequently was oxidised to the diketone (*2*) as described above for the natural compound. The physical properties except for optical activity of the products *1* (R=H) and *2* were identical to those of the tobacco-isolate and its oxidation product.

Partial syntheses of (9R)-9-acetoxy-4-megastigmen-3-one (9, R=Ac), (9R)-9-hydroxy-4-megastigmen-3-one (9, R=H) and 4-megastigmen-3,9-dione (10). (9*R*)-9-hydroxy-4,7*E*-megastigmadien-3-one [3] (*8*, R=H, 93 mg) was acetylated, selectively hydrogenated, hydrolyzed, and oxidized as described above. The products were indistinguishable from the natural alcohol, its acetate and oxidation product except for having somewhat lower specific rotations; see Table 1.

Epimerization of authentic (9R)-9-hydroxy-4-megastigmen-3-one (9, R=H). The authentic ketol *9* (R=H, 6.3 mg) was dissolved in MeOH, a small amount of $NaOCH_3$ (2 drops of a 3 % solution in MeOH) was added and the change in optical acitivity was followed. After about 10 h no further change in the activity could be observed; 436 nm: −5.2°, 456 nm: −3.8°, 578 nm: −3.5°, 589 nm: −3.2°.

Epimerization of natural (9R)-9-hydroxy-4-megastigmen-3-one (9, R=H): The natural ketol *9* (R=H, 5.7 mg) was treated as described above for the authentic ketol. Optical activity of the equilibrium mixture: 436 nm: −7.7°, 546 nm: −5.4°, 578 nm: −4.7°, 589 nm: −4.7°. Dilution with water and extraction with ether yielded 4.3 mg which were dissolved in $CHCl_3$ (1 ml): 436 nm: −7.7°, 546 nm: −6.0°, 578 nm: −5.1°, 589 nm: −3.3°.

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Added in proof: Comparison of the optical properties of the new tobacco constituent with those of blumenol C leaves no doubt about their identity (M. N. Galbraith, private communication). Dr. A. Demole, Firmenich SA, Geneva, has recently independently identified the title compound as a burley tobacco constituent (private communication).

REFERENCES

1. Aasen, A. J., Kimland, B. and Enzell, C. R. *Acta Chem. Scand. 25* (1971) 1481.
2. Aasen, A. J., Kimland, B. and Enzell, C. R. *Acta Chem. Scand. 27* (1973) 2107.
3. Hlubucek, J. R., Aasen, A. J., Kimland, B. and Enzell, C. R. *To be published.*
4. Kimland, B., Aasen, A. J. and Enzell, C. R. *Acta Chem. Scand. 26* (1972) 2177.
5. Culvenor, C. C. J. *Tetrahedron Lett.* (1966) 1091.
6. Aasen, A. J., Kimland, B., Almqvist, S.-O. and Enzell, C. R. *Acta Chem. Scand. 26* (1972) 2573.
7. Roberts, D. L. and Schumacher, J. N. *US Patent* 3,217,716, Nov. 16, 1965.
8. Galbraith, M. N. and Horn, D. H. S. *Chem. Commun.* (1972) 113.
9. Pousset, J.-L. and Poisson, J. *Tetrahedron Lett.* (1969) 1173.
10. Eugster, C. H., Buchecker, R., Tcharner, Ch., Uhde, G. and Ohloff, G. *Helv. Chim. Acta 52* (1969) 1729.
11. Buchecker, R., Hamm, P. and Eugster, C. H. *Chimia 25* (1971) 192.
12. Galbraith, M. N. and Horn, D. H. S. *Chem. Commun.* (1973) 566.
13. Weiss, G., Koreeda, M. and Nakanishi, K. *Chem. Commun.* (1973) 565.
14. Wright, H. E., Burton, W. W. and Berry, R. C. *Arch. Biochem. Biophys. 82* (1959) 107.
15. Demole, E. and Berthet, D. *Helv. Chim. Acta 55* (1972) 1866.
16. Brown, H. C., Garg, C. P. and Liu, K.-T. *J. Org. Chem. 36* (1971) 387.
17. Hershberg, E. B., Oliveto, E. P., Gerold, C. and Johnson, L. *J. Amer. Chem. Soc. 73* (1951) 5073.
18. Augustine, R. L. *Catalytic Hydrogenation*, Dekker, New York 1965.

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