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Tobacco Chemistry. 29. (7*S*)-10-Oxo-4ξ-methyl-7-isopropyl-5*E*-undecen-4-olide, a New Thunbergan-type Nor-isoprenoid Isolated from Greek *Nicotiana tabacum* L.

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The structure of a new C_{15}-lactone, (7*S*)-10-oxo-4ξ-methyl-7-isopropyl-5*E*-undecen-4-olide, isolated from Greek tobacco, has been determined mainly by ^{1}H NMR, ^{13}C NMR and high resolution MS, and its absolute configuration established by degradation to (2*S*)-5-oxo-2-isopropylhexanal. The carbon skeleton of the new compound indicates that it is derived from a thunbergane precursor and constitutes the first C_{15} nor-thunberganoid in tobacco.

Some 200 nor-isoprenoids have so far been encountered in tobacco leaves and tobacco smoke. Virtually all of these can be viewed as derived from either (i) carotenoids/aliphatic polyisoprenoids, (ii) labdanes/drimanes, or (iii) thunberganes, and are conveniently grouped accordingly.[1] They constitute a substantial amount of the tobacco essential oil and many of them are considered important for the tobacco flavour and aroma.[2] In the present communication** we present evidence for the structure (*1*) of a new lactone isolated from sun-cured Greek *Nicotiana tabacum* L. and evidently derived from a thunbergane precursor.

The presence of fifteen lines in the ^{13}C NMR spectrum and precise mass determination established that the new compound had the elemental composition $C_{15}H_{24}O_3$. The three oxygen atoms, as judged from IR, ^{13}C NMR, and ^{1}H NMR, were accomodated in a methyl ketone grouping (1716 cm^{-1}; δ_C 208.9, s; δ_H 2.1, 3 H, s) and a γ-lactone moiety (1777 cm^{-1}; δ_C 176.9, s). Of the additional three methyl groups revealed by the spectral data, two were present in an isopropyl group (1370 and 1386 cm^{-1}; δ_C 19.1, q, and 20.7, q; δ_H 0.82, 3 H, d, and 0.88, 3 H, d), and the third was linked to the γ-atom of the lactone ring, and, according to the low field chemical shift of its proton resonance (δ_H 1.5, 3 H, s) allylic to the *trans* disubstituted double bond present in the molecule (981 cm^{-1}; δ_C 131.6, d, and 134.1, d; δ_H 5.5, 2 H, m). Examination of the high resolution mass spectrum confirmed the presence of these structural features and implied structure *1* for the new compound. Thus, a doublet at *m/e* 209 ($C_{13}H_{21}O_2$ and $C_{12}H_{17}O_3$) was consistent with the occurrence of the acetyl and isopropyl groups, and peaks at *m/e* 153 ($C_{10}H_{17}O$), 99 ($C_5H_7O_2$), and 125 ($C_7H_9O_2$) were in accord with the presence of the 4-methyl-4-vinylene γ-lactone unit. Moreover, an ion at *m/e* 194 ($C_{12}H_{18}O_2$) demonstrated the loss of the elements of acetone from the molecular ion by a McLafferty reaction and hence, the attachment of the acetyl group to a methylene group. The remaining methylene group could be inserted in three ways giving 10-oxo-4-methyl-7-isopropyl-5*E*-undecen-4-olide (*1*), 10-oxo-4-methyl-8-isopropyl-5*E*-undecen-4-olide (*3*), or 9-oxo-4-methyl-7-isobutyl-5*E*-decen-4-olide (*4*) of which the two last possibilities were disfavoured by MS and ^{13}C NMR evidence. Thus, the presence of an $C_{11}H_{17}O_2^+$ ion is only readily rationalized on the basis of structure *1*, which on electron impact should undergo simple cleavage of the allylic C(7)—C(8) bond extending from the tertiary C(7).

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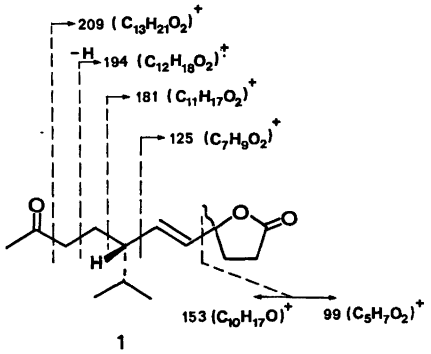

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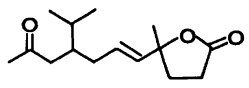

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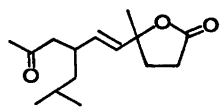

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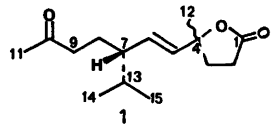
Similarly, the ^{13}C NMR data were consistent only with structure *1*, since in the case of structure *3* the resonance for the methylene carbon α to the keto group should occur at a lower field than observed (δ_C 41.9, t) because of the β-substitution, and in the case of structure *4* the signals due to the *geminal* methyl carbons, here incorporated in an isobutyl group, should occur at a lower field (δ_C 22 – 23) than observed (δ_C 19.1, q, and 20.7, q).[3,4] Moreover, the excellent agreement of relevant ^{13}C NMR signals observed for solanone (*2*) and 4-methyl-5-hexen-4-olide (*7*) with those of the tobacco isolate strongly supported the suggested structure: 10-oxo-4-methyl-7-isopropyl-5*E*-undecen-4-olide (*1*, see Table 1).*

Conclusive evidence for the presence of a 2-oxo-5-isopropylhex-1-ylidene moiety was obtained by the isolation of 5-oxo-2-isopropylhexanal (*5*) on ozonolytic degradation of the lactone. This ketoaldehyde (*5*) was optically active and exhibited the same positive rotatory power as the corresponding material (*5*) similarly prepared from solanone (*2*) thereby establishing the absolute configuration at C(7) as *S*.**

Additional evidence for the chemically unverified part of the molecule and for the *trans* configuration of the double bond was obtained by spectral examination of the triol diacetate

Table 1. ^{13}C chemical shifts (ppm relative to TMS) and assignments recorded for (7*S*)-10-oxo-4-ξ-methyl-7-isopropyl-5*E*-undecen-4-olide (*1*), and model compounds *2* and *7*.



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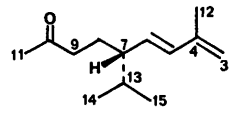

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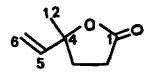

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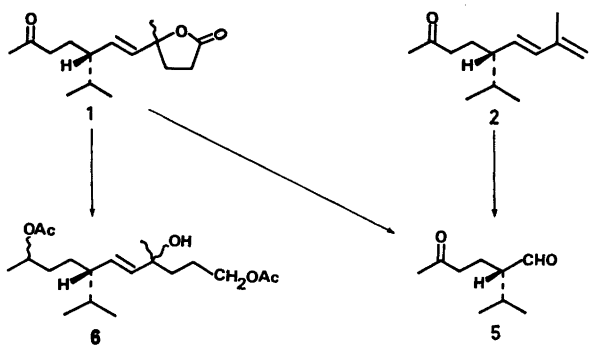
^{13}C assignment	Chemical shifts[a] (δ) and No. of lines[b] (n) 1 δ	n	2 δ	n	7 δ	n
C(1)	176.9	s			176.7	s
C(2)	29.1	t			28.7	t
C(3)	34.4	t	114.7	t	33.6	t
C(4)	85.4	s	141.8	s	85.4	s
C(5)	131.6[c]	d	132.2[c]	d	140.4	d
C(6)	134.0[c]	d	134.6[c]	d	113.4	t
C(7)	48.7	d	49.5	d		
C(8)	26.0	t	26.4	t		
C(9)	41.9	t	41.9	t		
C(10)	208.9	s	208.2	s		
C(11)	30.0	q	29.9	q		
C(12)	26.6	q	18.7	q	26.1	q
C(13)	31.9	d	32.5	d		
C(14)	19.1	q	19.3	q		
C(15)	20.7	q	20.8	q		

[a] Measured in proton noise-decoupled spectrum; [b] No. of lines observed in off-resonance proton-decoupled spectrum ('s' = singlet etc.); [c] Tentative.

* Dr. E. Demole, Firmenich SA, Geneve, has independently and simultaneously identified 10-oxo-4-methyl-7-isopropyl-5*E*-undecen-4-olide as a new constituent of Burley tobacco (private communication).

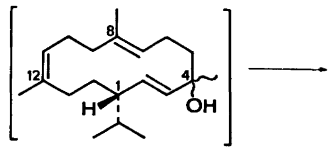
** Originally the L-configuration was erroneously attributed to solanone.[5] The correct *S* (or D)-configuration was later established through correlation with (2*S*)-5-oxo-2-isopropylhexanoic acid by Fukuzumi *et al.*[6] who, however, applied the sequence rules of Cahn, Ingold and Prelog,[7–9] (further exemplified by Weedon[10]) incorrectly by assigning the *R*-configuration to solanone.



(*6*; 3460 cm^{-1}; δ_H 4.1, 2 H, t, and 4.8, 1 H, m) prepared by $LiAlH_4$-reduction of the tobacco constituent (*1*) and subsequent acetylation under mild conditions. Thus, electron impact generated the structurally required fragments, notably the important M − 101 (74 %) produced by cleavage of the labile allylic C(3) − C(4) bond extending from the hydroxylated tertiary carbon, and its daughter ions M − 101 − 60 (100 %, m* 135.9) and M − 101 − 60 − 18 (42 %, m* 146.8) formed by consecutive losses of acetic acid and water. It exhibited IR absorption (979 cm^{-1}) characteristic of a *trans* disubstituted double bond and displayed, after addition of $Eu(dpm)_3$ to the ^1H NMR sample, a 16 Hz vicinal spin-spin coupling constant for the two olefinic protons constituting the AB-part of an ABX-system.

Shortage of material prevented configurational assignment of the second chiral centre, C(4). However, the CMR and ^1H NMR spectra (including those recorded after addition of shift reagent) indicated that the tobacco isolate was configurationally homogeneous as no additional resonances were observed; this, as previously found for the structurally related methyl 3-hydroxy-3-methyl-6-isopropyl-4*E*-octenoate,[11,12] would have been expected if two C(4)-epimers were present.

The structural relationship of this new lactone with the macrocyclic thunbergane diterpenoids, which are quite abundant in tobacco, places it in the growing family of volatile tobacco constituents assumed to be derived from such diterpenoids. A possible precursor, although as yet not detected in tobacco, is the hydrated thunbergene, thunberga-2,7-11-trien-4-ol (*8*)* which under the influence of oxygen and light could undergo oxidative cleavages of the C(7) − C(8) and C(11) − C(12) bonds furnishing the C_{15}-lactone. Most of the thunbergan-derived nor-isoprenoids previously encountered in tobacco are either C_{12}- or C_{13}-compounds and the new lactone therefore represents the first member of a new subgroup. Nine of the eleven thunbergan-diterpenoids found in tobacco [1] are hydroxylated and unsaturated at positions corresponding to those in the present lactone, and, being potential precursors, they might all possess the same *S*-configuration at the carbon atom carrying the isopropyl group.



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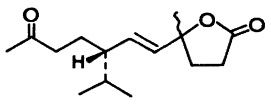

EXPERIMENTAL

^1H NMR, ^{13}C NMR (accuracy of δ_C: ± 0.05 ppm), IR, UV, and mass spectra were recorded on Varian XL-100, Digilab FTS-14, Beckmann DB-2A, and LKB 9000 instruments, respec-

* Nomenclature according to J. W. Rowe Oct. 1968; private communication.

tively. Rotations were measured on a Perkin-Elmer 141 instrument, and precise mass determinations were carried out at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm.

7(S)-10-Oxo-4-ξ-methyl-7-isopropyl-5E-undecen-4-olide (1, 35 mg) was isolated from a medium-volatile, neutral fraction [13] of an extract,[14] obtained from 295 kg sun-cured Greek *Nicotiana tabacum* L., employing liquid chromatography on silica gel and $AgNO_3$-impregnated silica gel. The fractionation of this medium-volatile material will be described elsewhere.[13] MS (20 eV): *m/e* 252 (M^+, 6), 43 (100), 121 (44), 107 (33), 99 (31), 93 (31), 95 (25), 81 (24), 109 (24), 97 (22), 71 (22), 123 (22), 135 (22), 149 (22), 151 (22), 111 (21), 194 (19), 55 (19), 69 (18), 119 (18), 125 (18), 83 (18), 137 (17), 134 (17), 139 (16), 112 (12), 105 (11), 108 (11), 133 (11), 122 (9), 179 (9), 181 (9), 161 (9), 163 (8), 59 (8), 234 (8); accurate mass determinations: $C_{15}H_{24}O_3$, found 252.1726, calc. 252.1725; $C_{13}H_{21}O_2$, found 209.1538, calc. 209.1541; $C_{12}H_{17}O_3$, found 209.1175, calc. 209.1178; $C_{12}H_{18}O_2$, found 194.1302, calc. 194.1307; $C_{11}H_{17}O_2$, found 181.1219, calc. 181.1228; $C_{10}H_{17}O$, found 153.1277, calc. 153.1279; $C_7H_9O_2$, found 125.0595, calc. 125.0602; $C_5H_7O_2$, found 99.0450, calc. 99.0446; ν_{max} (film): 2965 (s), 2948 (s), 2876 (m), 1777 (s), 1716 (s), 1386 (shoulder), 1370 (m), 1240 (m), 1205 (m), 1169 (m), 1141 (m), 1078 (m), 981 (m), 932 (m); δ_H ($CDCl_3$): 0.82 (3 H, d, *J* 6.5 Hz), 0.88 (3 H, d, *J* 6.5 Hz), 1.50 (3 H, s), *ca.* 2.0–2.8 (*ca.* 4 H, m), 2.12 (3 H, s), 5.5 (2 H, m); addition of Eu(dpm)$_3$: *r* (relative induced shifts) 0.57 and 0.65 [C(7)-CH(C*H*$_3$)$_2$, overlapping doublets], 1.0 [C(4)C*H*$_3$, s], 1.24 [C(5)*H*=C(6)*H*, m], 3.35 [C(11)*H*$_3$, s]; δ_C ($CDCl_3$): see Table 1; $[\alpha]^{20}$ +1.3° (589 nm), +1.9° (578), +2.4° (546), +5.7° (436), (*c* 1.6, $CHCl_3$).

4-Hydroxy-1,10-diacetoxy-4-methyl-7-isopropyl-5E-undecene (6): (7*S*)-10-oxo-4-ξ-methyl-7-isopropyl-5*E*-undecen-4-olide (*1,* 10 mg) dissolved in dry ether (3 ml) was added dropwise to a solution of $LiAlH_4$ (8 mg) in dry ether (20 ml) and the mixture stirred for 1 h at room temperature. Moist ether, followed by water (10 ml) was added to the reaction mixture and the products extracted with ether. Removal of the solvent left a homogeneous (TLC) product (13 mg) which was acetylated without further purification. The residue was dissolved in dry pyridine (1.5 ml) and acetic anhydride (0.3 ml) and kept at ambient temperature for 6 h. Excess anhydride was destroyed with a few drops methanol, water (10 ml) was added and the acetate extracted with ether. The extract was washed with H_2SO_4 (dil.), water, $NaHCO_3$, water, dried and evaporated leaving the diacetate as a colourless oil which was chromatographed on a silica gel column. The diacetate (9 mg) was eluted with 50 % ether/pentane. MS (20 eV, ion source temp. 120 °C): *m/e* 342 (M^+, not visible), 181 (100), 241 (74), 43 (42), 111 (42), 163 (42), 137 (29), 71 (33), 123 (28), 264 (21), 182 (15); metastables were observed at *m/e* 215.1, 185.0, 135.9, 146.8, and 172.6, respectively, corresponding to the transitions 324 (M−18)→264 (M−18−60)→221 (M−18−60−43), 241 (M−101)→181 (M−101−60)→163 (M−101−60−18), and 207 (M−15−2×60)→189 (M−15−2×60−18); ν_{max} (film): 3460 (broad), 2959 (m), 2936 (shoulder), 2871 (m), 1739 (s), 1455 (w), 1383 (shoulder), 1372 (m), 1368 (m), 1247 (s), 1130 (w), 1031 (m), 979 (w), 952 (w); δ_H ($CDCl_3$): 0.91 (3 H, d, *J* 6.5 Hz), 0.96 (3 H, d, *J* 6.5 Hz), 1.19 (3 H, d, *J* 6 Hz), 1.30 (3 H, s), 2.01 (3 H, s), 2.03 (3 H, s), 4.07 (2 H, t with further splittings), *ca.* 4.83 (1 H, m), 5.43 (2 H, m); irradiations at δ 1.19 and 1.58, respectively, simplified the multiplet at δ *ca.* 4.83 to a distorted triplet and the multiplet at δ 4.07 to a broad singlet; irradiation at δ *ca.* 4.83 collapsed the doublet at δ 1.19 to a singlet; addition of Eu(dpm)$_3$: *r* (relative induced shifts) 0.18 and 0.23 [C(7)CH(C*H*$_3$)$_2$, m], 0.42 and 0.46 [C(11)*H*$_3$, 2×d], 0.65 and 0.67 [2×COC*H*$_3$], 1.0 [C(1)*H*$_2$, t], 1.23 [C(10)*H*, m], 1.26 [C(6)*H*, m], 1.81 [C(5)*H*, d, *J* 16 Hz], 1.75 and 1.82 [C(4)C*H*$_3$, 2×s]; decoupling experiments performed on the Eu(dpm)$_3$-treated sample: irradiation at δ 5.80 [C(10)*H*, *r* 1.23] collapsed the two doublets at δ 1.52 (*r* 0.42) and δ 1.55 (*r* 0.46), respectively, to a broad singlet [C(11)H$_2$]; irradiation at δ 1.54 sharpened the multiplet at δ 5.80 [C(10)*H*].

Ozonolysis of (7S)-10-oxo-4ξ-methyl-7-isopropyl-5E-undecen-4-olide (1). The natural lactone (*1,* 15 mg) dissolved in CH_2Cl_2 (15 ml) and pyridine (0.1 ml) was treated with excess ozone at −65 °C. Zinc-powder (500 mg) and acetic acid (2 ml) were added and the mixture stirred 35 min, during which the temperature was slowly raised to 20 °C. The solution was decanted, the reaction vessel with its residue was washed with CH_2Cl_2, and the combined solutions washed four times with water. Removal of the solvent left a slightly yellow oil which was chromatographed on silica gel furnishing pure (2*S*)-5-oxo-2-isopropylhexanal (*5,* 2 mg); MS: *m/e* 156 (M^+, 2), 43 (100), 58 (75), 41 (35), 71 (23), 69 (22), 55 (19), 95 (18), 70 (10), 59 (10), 96 (10), 86 (9), 110 (9), 138 (8), 85 (8), 81 (8), 123 (7); ν_{max} (film): 2967 (m), 2935 (m), 2879 (w), 2720 (w), 1722 (s), 1719 (s), 1372(m), 1170 (m); lit.:[15] ν_{max} (film): 1709 (C=O); δ_H ($CDCl_3$) 0.97 (3 H, d, *J* 7 Hz), 1.00 (3 H, d, *J* 7 Hz), 2.13 (3 H, s), 2.3–2.6 (2 H, m), 9.63 (1 H, d, *J* 2 Hz); lit.:[15] δ 1.0 (isopropyl methyls, *J* 6 Hz), 2.07 (acetyl methyl);$[\alpha]^{20}$ +47° (589 nm), +49° (578), +60° (546), +165° (436), +415° (365), (*c* 0.2, $CHCl_3$). The ketoaldehyde did not separate from the corresponding ketoaldehyde *5* prepared from solanone (*vide infra*) when co-injected on a glass capillary GC-column.

Ozonolysis of solanone. Solanone ((5*S*)-8-methyl-5-isopropyl-6*E*,8-nonadien-2-one, 18mg); $[\alpha]^{20}$ +16.7° (589 nm), +17.1° (578), +20.6° (546), +45.6° (436), +114.3° (365), (*c* 0.62, $CHCl_3$);

lit.: $[\alpha]_D^{23}$ + 13.6° (neat),[5] $[\alpha]_D^{28}$ + 14.2° ($CHCl_3$);[6] previously procured from the same tobacco (fraction B3),[14,16] was ozonolyzed and the ketoaldehyde *5* (6.2 mg) isolated as described above; $[\alpha]^{20}$ +43.7° (589 nm), +45.9° (578), +55.6° (546), +133.3° (436), +364.8 (365), (*c* 0.54, $CHCl_3$); the NMR, IR and mass spectra were indistinguishable from those of the ketoaldehyde obtained from the tobacco constituent.

Model compounds. Solanone (*2*), isolated from Greek tobacco,[14,16] and 4-methyl-5-hexen-4-olide (*7*), obtained in almost quantitative yield by CrO_3/AcOH oxidation of linalool oxide, was used for comparison of ^{13}C NMR data, see Table 1.

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