# New Lactones from Tobacco†

Tore Pettersson, Ann-Marie Eklund, and Inger Wahlberg*

Reserca AB, S-118 84 Stockholm, Sweden

Thirteen lactones (1–13) have been isolated from an extract of sun-cured leaves of Greek tobacco by using HPLC methods. All but four of these (1–9) are new natural products. They have been identified as 3-(4-methyl-1-pentyl)-2-buten-4-olide (1), 3-isopropyl-2-penten-4-olide (2), 3-ethyl-4-methyl-2-penten-4-olide (3), 4-methyl-3-(3-oxo-1-butyl)-2-penten-4-olide (4), 3-methyl-7-oxo-2-octen-4-olide (5), 4-(5-methyl-2-furyl)pentan-4-olide (6), (3*R**,4*R**,7*R**)-3,7-epoxy-4,8-dimethyl-8-nonen-4-olide (7), 3-isopropyl-2,4-pentadien-5-olide (8), and (1*S*,7*R*)-4-oxa-7,11,11-trimethylbicyclo[5.4.0]undecan-3-one (9) by spectral methods, 2D NMR being a helpful tool. The syntheses of two of the lactones (1 and 9) are reported, and the plausible biogenesis of the new lactones is discussed. (*Z*)-6-Nonen-4-olide (10) and 5-methyl-4-hexanolide (11), now also isolated, are new to tobacco, while two 3-methyl-4-pentanolides (12 and 13), previously known as tobacco constituents, have now been identified as the 3*R*,4*R*- and 3*R*,4*S*-isomers by comparison of their optical rotations and spectral data with those of corresponding synthetic samples.

## INTRODUCTION

More than 80 lactones have been reported as tobacco constituents to date. Among these are α-levantenolide (14) and the $C_{20}$ lactone 15, diterpenoids of the labdane and cembrane classes, respectively (Giles and Schumacher, 1961; Wahlberg et al., 1986). Ambreinolide (16) and the carboacyclic 17 are lactone-containing compounds classified as degraded labdanoids and degraded cembranoids, respectively (Schumacher and Vestal, 1974; Aasen et al., 1975), while dihydroactinidiolide (18) and loliolide (19) are well-known lactones formed via biodegradation of carotenoids (Bailey et al., 1968; Behr et al., 1979). The lactones of tobacco also include structurally simple compounds of fatty acid origin such as 4-octanolide (20), 5-decanolide (21), and the macrocyclic 15-pentadecanolide (22) (Schumacher and Vestal, 1974; Fujimori et al., 1976; Demole and Enggist, 1978). Coumarin (23) and scopoletin (24) that derive from shikimic acid are also present in tobacco (Fujimori et al., 1976; Roberts and Rohde, 1972).

We now report the isolation by HPLC of 13 lactones (1–13) from an extract of sun-cured leaves of Greek tobacco. Eleven of these are new to tobacco (1–11), and nine are new natural products (1–9).

## EXPERIMENTAL PROCEDURES

**Instruments.** Gas chromatographical analyses for enantiomeric purity were performed on a Hewlett-Packard Model 5880A instrument equipped with a Chrompack CP Cyclodex B 236M column. The injection temperature was 100 °C, and the column was kept at 100 °C for 1 min and then programmed to 230 °C with a rate of 1 °C/min. High-performance liquid chromatography was carried out using Waters 6000A or Delta Prep 3000 solvent delivery systems, Waters U6K injectors, and Waters R-401 or R-403 differential refractometers. Melting points, optical rotations, and infrared spectra were recorded on a Leitz Wetzlar instrument, a Perkin-Elmer 241 polarimeter, and a Perkin-Elmer FT-IR 1725X spectrometer, respectively. NMR spectra were obtained on a Varian XL-300 instrument and mass spectra on a Kratos MS 50 Stereo DS 55 SM/DS 55 S mass spectrometer–computer system.

**Isolation.** 3-(4-Methyl-1-pentyl)-2-buten-4-olide (1, 0.7 mg), 3-isopropyl-2-penten-4-olide (2, 2.9 mg), 3-ethyl-4-methyl-2-penten-4-olide (3, 1.7 mg), 4-(5-methyl-2-furyl)pentan-4-olide (6, 6.6 mg), (3*R**,4*R**,7*R**)-3,7-epoxy-4,8-dimethyl-8-nonen-4-olide (7, 0.6 mg), 3-isopropyl-2,4-pentadien-5-olide (8, 1.6 mg), (1*S*,7*R*)-4-oxa-7,11,11-trimethylbicyclo[5.4.0]undecan-3-one (9, 4.8 mg), (*Z*)-6-nonen-4-olide (10, 10.9 mg), 5-methyl-4-hexanolide (11, 1.8 mg), (3*R*,4*R*)-3-methyl-4-pentanolide (12, 0.7 mg), and the corresponding 3*R*,4*S*-isomer 13 (0.8 mg) were all isolated from fraction B7 and 4-methyl-3-(3-oxo-1-butyl)-2-penten-4-olide (4, 1.6 mg) and 3-methyl-7-oxo-2-octen-4-olide (5, 0.9 mg) from fraction A3 of an $Et_2O$ extract of 295 kg of sun-cured Greek tobacco by chromatography over silica gel using hexane/EtOAc as the eluent (Kimland et al., 1972) followed by HPLC using columns packed with Partisil M9 PAC (hexane/EtOAc 80:20), Spherisorb 5 (hexane/EtOAc 60:40), and Spherisorb 5 nitrile (hexane/EtOAc 60:40).

3-(4-Methyl-1-pentyl)-2-buten-4-olide (1) was an oil: found $M^{\bullet+}$ 168.1125, calcd for $C_{10}H_{16}O_2$ 168.1150; IR ($CCl_4$) 1784, 1752, 1641, 1167, 1136, 1038, and 882 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 0.90 (d, $J$ = 6.7 Hz, H-9 and H-10), 1.24 (m, H-7a and H-7b), 1.58 (m, H-6a, H-6b, and H-8), 2.39 (br t, $J$ = 7.8 Hz, H-5a and H-5b), 4.74 (dt, $J$ = 0.6 and 1.6 Hz, H-4a and H-4b), and 5.84 (quintet, $J$ = 1.6 Hz, H-2); $^{13}C$ NMR ($CDCl_3$) δ 22.5 (C-9 and C-10), 25.1 (C-6), 27.8 (C-8), 28.8 (C-5), 38.4 (C-7), 73.1 (C-4), 115.4 (C-2), 170.6 (C-3), and 174.2 (C-1); MS [$m/z$ (%, composition)] 168 (2, M), 153 (1), 139 (3, $C_9H_{15}O$), 137 (3, $C_9H_{13}O$), 125 (6, $C_7H_9O_2$), 108 (78, $C_8H_{12}$), 98 (38, $C_5H_6O_2$), 95 (18, $C_7H_{11}$ and $C_6H_7O$), 82 (17, $C_6H_{10}$ and $C_5H_6O$), 81 (15, $C_6H_9$ and $C_5H_5O$), 70 (31, $C_5H_{10}$ and $C_4H_6O$), 69 (59, $C_5H_9$), 67 (26, $C_5H_7$ and $C_4H_3O$), 55 (63, $C_4H_7$ and $C_3H_3O$), 43 (100, $C_3H_7$ and $C_2H_3O$), and 41 (70, $C_3H_5$).

3-Isopropyl-2-penten-4-olide (2) was an oil: $[\alpha]_D$ −7.3° (*c* 0.11, $CHCl_3$); found $M^{\bullet+}$ 140.0838, calcd for $C_8H_{12}O_2$ 140.0838; IR ($CCl_4$) 1765, 1635, 1385, 1375, 1170, 950, and 860 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.16 (d, $J$ = 6.8 Hz)/1.25 (d, $J$ = 6.8 Hz) (H-7/H-8), 1.45 (d, $J$ = 6.8 Hz, H-5), 2.57 (d septet, $J$ = 1.5 and 6.8 Hz, H-6), 5.03 (dq, $J$ = 1.5 and 6.8 Hz, H-4), and 5.75 (t, $J$ = 1.5, H-2); MS [$m/z$ (%, composition)] 140 (2, M), 125 (5, $C_7H_9O_2$), 111 (3, $C_7H_{11}O$), 98 (51, $C_5H_6O_2$), 97 (100, $C_6H_9O$ and $C_5H_5O_2$), 81 (22, $C_6H_9$ and $C_5H_5O$), 69 (22, $C_5H_9$), 67 (17, $C_5H_7$), 55 (15, $C_4H_7$ and $C_3H_3O$), 53 (32, $C_4H_5$), 43 (76), and 41 (83).

3-Ethyl-4-methyl-2-penten-4-olide (3) was an oil: found $M^{\bullet+}$ 140.0825, calcd for $C_8H_{12}O_2$ 140.0837; IR ($CCl_4$) 1760, 1640, 1380, 1370, 1250, 980, 935, and 865 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.24 (t, $J$ = 7.3 Hz, H-8), 1.45 (s, H-5 and H-6), 2.28 (dq, $J$ = 1.9 and 7.3 Hz, H-7a and H-7b), and 5.70 (t, $J$ = 1.9 Hz, H-2); MS [$m/z$ (%, composition)] 140 (3, M), 125 (83, $C_7H_9O_2$), 111 (2, $C_6H_7O_2$), 97 (100, $C_6H_9O$), 82 (17, $C_5H_6O$), 67 (3, $C_4H_3O$), 59 (3, $C_3H_7O$), 54 (17, $C_4H_8$), and 43 (91).

4-Methyl-3-(3-oxo-1-butyl)-2-penten-4-olide (4) was an oil: found $M^{\bullet+}$ 182.0898, calcd for $C_{10}H_{14}O_3$ 182.0943; IR ($CHCl_3$) 1750, 1725, 1645, 1375, 1365, 1160, 985, and 950 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.48 (s, H-5 and H-6), 2.24 (s, H-10), 2.53 (m, H-7a and

* Author to whom correspondence should be addressed.

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H-7b), 2.81 (m, H-8a and H-8b), and 5.61 (t, $J$ = 1.8 Hz, H-2); $^{13}$C NMR ($CDCl_3$) $\delta$ 20.8 (C-7), 24.9 (C-5 and C-6), 30.0 (C-10), 40.2 (C-8), 87.4 (C-4), 113.8 (C-2), 171.8 (C-1), 176.3 (C-3), and 205.8 (C-9); MS [$m/z$ (%, composition)] 182 (1, M), 167 (7, $C_9H_{11}O_3$), 140 (42, $C_8H_{12}O_2$), 125 (23, $C_7H_9O_2$), 111 (9, $C_7H_{11}O$ and $C_6H_7O_2$), 97 (6, $C_6H_9O$), 96 (5, $C_6H_8O$), 95 (6, $C_7H_{11}$ and $C_6H_7O$), 81 (5, $C_6H_9$ and $C_5H_5O$), 67 (3, $C_5H_7$ and $C_4H_3O$), 53 (7, $C_4H_5$), and 43 (100).

3-Methyl-7-oxo-2-octen-4-olide (**5**) was an oil: $[\alpha]_D$ 0° ($c$ 0.07, $CHCl_3$); found $M^{\bullet+}$ 168.0782, calcd for $C_9H_{12}O_3$ 168.0787; IR ($CHCl_3$) 1760, 1715, 1650, 980, and 855 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.63 (dddd, $J$ = 5.1, 7.6, 8.9, and −14.6 Hz, H-5a), 2.09 (dd, $J$ = 0.8 and 1.5 Hz, H-9), 2.18 (s, H-8), 2.29 (ddt, $J$ = 3.2, 7.6, and −14.6 Hz, H-5b), 2.60 (ddd, $J$ = 5.1, 7.6, and −18.6 Hz, H-6a), 2.70 (dt, $J$ = 7.6 and −18.6 Hz, H-6b), 4.86 (dddq, $J$ = 0.8, 1.5, 3.2, and 8.9 Hz, H-4), and 5.82 (quintet, $J$ = 1.5 Hz, H-2); $^{13}$C NMR ($CDCl_3$) $\delta$ 13.9 (C-9), 25.9 (C-5), 30.1 (C-8), 38.1 (C-6), 83.3 (C-4), 117.0 (C-2), 168.6 (C-3), 172.1 (C-1), and 207.3 (C-7); MS [$m/z$ (%, composition)] 168 (13, M), 140 (13, $C_8H_{12}O_2$ and $C_7H_8O_3$), 126 (15, $C_7H_{10}O_2$), 125 (8, $C_7H_9O_2$), 111 (70, $C_6H_7O_2$), 98 (82, $C_5H_6O_2$), 97 (36, $C_6H_9O$ and $C_5H_5O_2$), 83 (4, $C_6H_{11}$ and $C_5H_7O$), 79 (4, $C_6H_7$), 69 (30, $C_5H_9$ and $C_4H_5O$), 58 (10, $C_3H_6O$), 55 (13, $C_4H_7$ and $C_3H_3O$), and 43 (100, $C_2H_3O$).

4-(5-Methyl-2-furyl)pentan-4-olide (**6**) was an oil: $[\alpha]_D$ −1.9° ($c$ 0.52, $CHCl_3$); found $M^{\bullet+}$ 180.0806, calcd for $C_{10}H_{12}O_3$ 180.0786; IR ($CCl_4$) 1780, 1560, 1245, 1200, 1130, 1025, and 935 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.76 (s, H-5), 2.28 (d, $J$ = 1.0, H-10), 5.90 (dq, $J$ = 1.0 and 3.2 Hz, H-8), and 6.20 (d, $J$ = 3.2 Hz, H-7); MS [$m/z$ (%, composition)] 180 (57, M), 165 (63, $C_9H_9O_3$), 136 (36, $C_9H_{12}O$), 135 (26, $C_9H_{11}O$), 125 (100, $C_7H_9O_2$), 121 (38, $C_8H_9O$), 109 (84, $C_6H_5O_2$), 93 (15, $C_7H_9$ and $C_6H_5O$), 91 (14, $C_7H_7$), 77 (14, $C_6H_5$), 65 (8, $C_5H_5$), 55 (19, $C_3H_3O$), 53 (20, $C_4H_5$), and 43 (93, $C_2H_3O$).

(3*R**,4*R**,7*R**)-3,7-Epoxy-4,8-dimethyl-8-nonen-4-olide (**7**) was an oil: $[\alpha]_D$ 0° ($c$ 0.03, $CHCl_3$); found $M^{\bullet+}$ 196.1080, calcd for $C_{11}H_{16}O_3$ 196.1099; IR ($CCl_4$) 1791, 1181, 1119, 1086, and 943 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.32 (s, H-11), 1.73 (m, H-10), 2.54 (d, $J$ = −17.6 Hz, H-2a), 2.89 (dd, $J$ = 4.3 and −17.6 Hz, H-2b), 3.73 (br d, $J$ = 9.3 Hz, H-7), 4.09 (d, $J$ = 4.3 Hz, H-3), 4.86 (m, H-9a), and 4.97 (m, H-9b); $^{13}$C NMR ($C_6D_6$) $\delta$ 18.5 (C-10), 24.9/25.2 (C-6/C-11), 32.3 (C-5), 38.0 (C-2), 77.6/78.5 (C-3/C-7), 80.2 (C-4), 111.1 (C-9), 145.2 (C-8), and 160.8 (C-1); MS [$m/z$ (%, composition)] 196 (44, M), 181 (46, $C_{10}H_{13}O_3$), 153 (18, $C_9H_{13}O_2$), 121 (12, $C_8H_9O$ and $C_9H_{13}$), 111 (17, $C_6H_7O_2$ and $C_7H_{11}O$), 98 (15, $C_5H_6O_2$ and $C_6H_{10}O$), 81 (16, $C_6H_9$), 69 (22, $C_4H_5O$ and $C_5H_9$), 55 (27, $C_3H_3O$ and $C_4H_7$), and 43 (100, $C_2H_3O$ and $C_3H_7$).

3-Isopropyl-2,4-pentadien-5-olide (**8**) was an oil: found $M^{\bullet+}$ 138.0688, calcd for $C_8H_{10}O_2$ 138.0681; IR ($CCl_4$) 1742, 1640, 1553, 1386, 1370, and 1251 cm$^{-1}$; $^1$H NMR ($C_6D_6$) $\delta$ 0.62 (d, $J$ = 6.8 Hz, H-7 and H-8), 1.85 (d septet, $J$ = 1.0 and 6.8 Hz, H-6), 5.11 (dd, $J$ = 1.8 and 5.5 Hz, H-4), 5.83 (dt, $J$ = 1.0 and 1.8 Hz, H-2), and 6.53 (dd, $J$ = 1.0 and 5.5 Hz, H-5); MS [$m/z$ (%, composition)] 138 (39, M), 123 (3, $C_7H_7O_2$), 110 (20, $C_7H_{10}O$), 95 (100, $C_6H_7O$), 81 (10, $C_6H_9$ and $C_5H_5O$), 67 (32, $C_5H_7$ and $C_4H_3O$), 53 (12, $C_4H_5$), and 41 (34).

(1*S*,7*R*)-4-Oxa-7,11,11-trimethylbicyclo[5.4.0]undecan-3-one (**9**): mp 90.5–93.5 °C; $[\alpha]_D$ −10° ($c$ 0.14, $CHCl_3$); found $M^{\bullet+}$ 210.1619, calcd for $C_{13}H_{22}O_2$ 210.1620; IR ($CCl_4$) 1735, 1290, 1130, and 1080 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.80 (s, H-13), 0.98 (s, H-12), 1.08 (t, $J$ = 0.9 Hz, H-14), 1.36 (dd, $J$ = 0.9 and 10.3 Hz, H-1), 1.48 (dd, $J$ = 5.5 and −14.4 Hz, H-6a), 1.65 (ddd, $J$ = 2.0, 11.1, and −14.4 Hz, H-6b), 2.48 (dd, $J$ = 0.9 and −13.9 Hz, H-2a), 2.61 (dd, $J$ = 10.3 and −13.9 Hz, H-2b), 4.10 (ddd, $J$ = 2.0, 5.5, and −13.3 Hz, H-5a), and 4.35 (dd, $J$ = 11.1 and −13.3 Hz, H-5b); $^{13}$C NMR ($CDCl_3$) $\delta$ 18.4 (C-9), 18.9 (C-14), 20.9 (C-13), 31.5 (C-2), 32.9 (C-12), 34.4/36.1 (C-7/C-11), 41.6/41.9 (C-8/C-10), 47.9 (C-6), 50.3 (C-1), 64.8 (C-5), and 176.6 (C-3); MS [$m/z$ (%, composition)] 210 (8, M), 195 (8, $C_{12}H_{19}O_2$), 177 (5, $C_{12}H_{17}O$), 167 (22, $C_{10}H_{15}O_2$), 153 (33, $C_{10}H_{17}O$), 138 (20, $C_{10}H_{18}$), 123 (27), 115 (31, $C_6H_{11}O_2$), 109 (28), 107 (18, $C_8H_{11}$), 96 (52, $C_7H_{12}$ and $C_6H_8O$), 95 (40, $C_7H_{11}$ and $C_6H_7O$), 81 (71, $C_6H_9$ and $C_5H_5O$), 69 (78, $C_5H_9$), 68 (40, $C_5H_8$), 67 (45, $C_5H_7$), 55 (62, $C_4H_7$ and $C_3H_3O$), and 41 (100, $C_3H_5$ and $C_2HO$).

(*Z*)-6-Nonen-4-olide (**10**) was an oil: $[\alpha]_D$ 0° ($c$ 0.33, $CHCl_3$); the IR, $^1$H NMR, and mass spectra were identical with those published for an authentic sample (Maurer and Hauser, 1982).

5-Methyl-4-hexanolide (**11**) was an oil: $[\alpha]_D$ 0° ($c$ 0.13, $CHCl_3$); the IR, $^1$H NMR, and mass spectra were identical with those published for an authentic sample (Timmer et al., 1975).

(3*R*,4*R*)-3-Methyl-4-pentanolide (**12**) was an oil: $[\alpha]_D$ +54° ($c$ 0.07, $CDCl_3$); the optical rotation and $^1$H and $^{13}$C NMR spectra agreed well with published data for a synthetic sample (Byström et al., 1981; Mosandl and Gunther, 1989).

(3*R*,4*S*)-3-Methyl-4-pentanolide (**13**) was an oil: $[\alpha]_D$ −60° ($c$ 0.08, $CHCl_3$); the optical rotation and $^1$H and $^{13}$C NMR and mass spectra agreed well with published data for a synthetic sample (Byström et al., 1981; Najera et al., 1984; Mosandl and Gunther, 1989).

**Synthesis.** *Preparation of 3-(4-Methyl-1-pentyl)-2-buten-4-olide* (**1**). A solution of 22 mg of 3-(4-methyl-3-penten-1-yl)-2-buten-4-olide (**25**), prepared according to the method of Corey and Schmidt (1980), and a catalytic amount of Pd/C (5%) in 5 mL of pentane was treated with hydrogen for 7.5 h. The reaction mixture was filtered and evaporated to give 9.1 mg of a residue. Purification by HPLC (Spherisorb 5 CN, hexane/EtOAc 60:40) gave as the main product 3.4 mg of 3-(4-methyl-1-pentyl)-2-buten-4-olide, the IR, $^1$H NMR, and mass spectra of which were identical with those of the naturally occurring **1**.

*Oxidation of 2-[5-Methyl-5-(5-methyl-2-furyl)tetrahydro-2-furyl]-2-propanol* (**26**). To a solution of 45 mg of pyridinium chlorochromate in 2 mL of dry dichloromethane was added a solution of 11.2 mg of 2-[5-methyl-5-(5-methyl-2-furyl)tetrahydro-2-furyl]-2-propanol (**26**) (Almqvist et al., 1974) in 2 mL of dry dichloromethane. The reaction mixture was boiled under reflux for 5 h and filtered through a short pad of Celite and silica gel. The eluate obtained was concentrated and shown by GC–MS to consist of a mixture of compounds. One of the minor products gave a mass spectrum identical with that of the naturally occurring **6**.

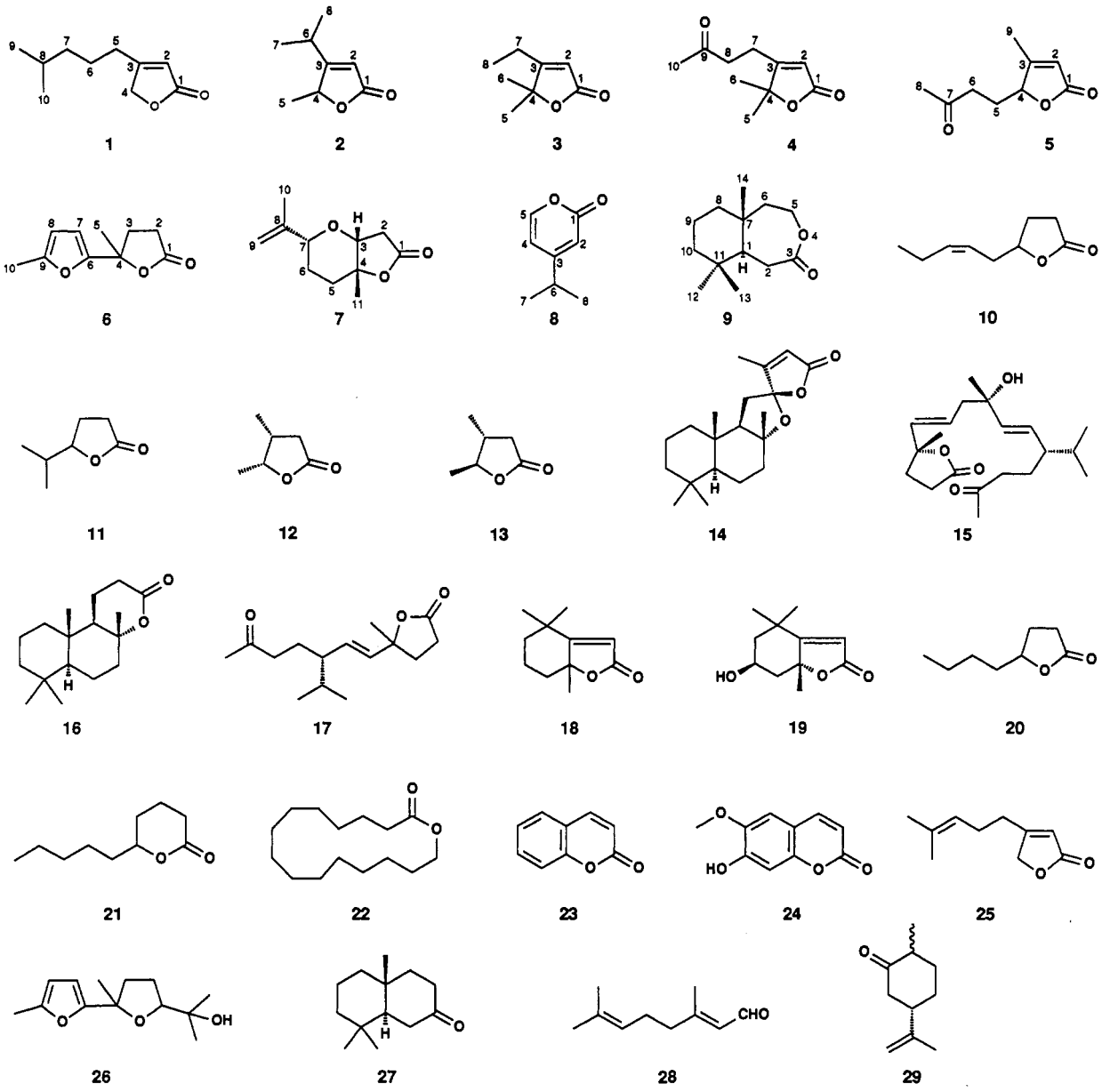
*Preparation of (1S,7R)-4-Oxa-7,11,11-trimethylbicyclo[5.4.0]-undecan-3-one* (**9**). To a solution of 125 mg of sodium acetate in acetic acid was added 200 mg of (1*S*,6*R*)-6,10,10-trimethylbicyclo[4.4.0]decan-3-one (**27**), which was prepared as described by Jansen et al. (1989). After addition of 350 $\mu$L of aqueous peracetic acid (32%), the reaction mixture was stirred for 6 days at ambient temperature. Workup and filtration through a silica bed using diethyl ether/pentane (1:1) as the eluent and subsequent separation by HPLC (Spherisorb 5, hexane/EtOAc 75:25) gave 50 mg of (1*S*,7*R*)-4-oxa-7,11,11-trimethylbicyclo[5.4.0]undecan-3-one which was identical (mp, optical rotation, and IR, $^1$H, $^{13}$C NMR and mass spectra) with the new tobacco lactone **9**.

## RESULTS

The first new compound (**1**), $C_{10}H_{16}O_2$, is an $\alpha,\beta$-unsaturated $\gamma$-lactone (IR bands at 1784, 1752, and 1641 cm$^{-1}$), which was identified as 3-(4-methyl-1-pentyl)-2-buten-4-olide with the aid of NMR results. H-2 resonated as a narrowly split quintet at $\delta$ 5.84, the two protons attached to C-4 as a doublet of triplets at $\delta$ 4.74, and the two methyl groups attached to C-8 as overlapping three-proton doublets at $\delta$ 0.90 in the $^1$H NMR spectrum. Correlations were found in the COSY spectrum between H-2 and the protons at C-4 and C-5 and between the protons at C-4 and those at C-5.

Confirmatory structural evidence was provided by synthesis. This involved the conversion of geranial (**28**) to the corresponding *O*-trimethylsilylcyanohydrin and subsequent treatment with pyridinium dichromate in dimethylformamide according to the procedure described by Corey and Schmidt (1980). The lactone obtained (**25**) was selectively hydrogenated using Pd/C as the catalyst to give **1**.

The second new compound (**2**), $C_8H_{12}O_2$, was identified as a 3-isopropyl-2-penten-4-olide from its spectral data. Thus, H-2 appears as a narrowly split triplet at $\delta$ 5.75 in the $^1$H NMR spectrum and is long-range-coupled both to H-4 at $\delta$ 5.03 and to H-6 at $\delta$ 2.57. H-4, in turn, is coupled to H-5 which resonates as a three-proton doublet at $\delta$ 1.45,




while the methyl groups of the isopropyl group, H-7 and H-8, give rise to three-proton doublets present at $\delta$ 1.16/1.25.

3-Isopropyl-2-penten-4-olide (**2**) has previously been reported as a synthetic product. Physical and spectral data for **2** were not, however, given in this paper (Larock et al., 1990).

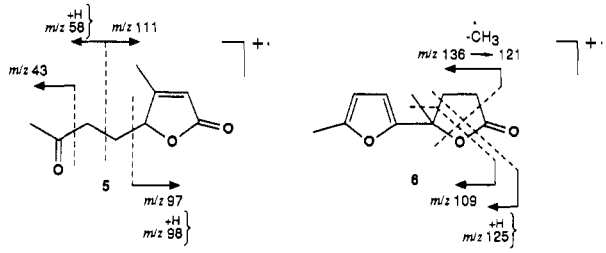
The third new compound (**3**), $C_8H_{12}O_2$, is also an $\alpha,\beta$-unsaturated $\gamma$-lactone (IR bands at 1760 and 1640 $cm^{-1}$). Its $^1H$ NMR spectrum revealed the presence of three methyl groups, of which two, resonating as a six-proton singlet at $\delta$ 1.45, are evidently attached to a fully substituted oxygen-carrying carbon atom. The remaining methyl group (a triplet at $\delta$ 1.24) forms part of an ethyl substituent deduced to be linked to the $\beta$-carbon atom of the unsaturated lactone system, because of the long-range coupling between the methylene protons of the ethyl group and the olefinic proton resonating at $\delta$ 5.70. Compound **3** was hence identified as 3-ethyl-4-methyl-2-penten-4-olide. This assignment was reinforced by a comparison of its $^1H$ NMR data with those published for the synthetic 3,4-dimethyl-2-penten-4-olide (Smith et al., 1981).

It was concluded from the IR (1750 and 1645 $cm^{-1}$) and $^1H$ NMR spectra [$\delta$ 5.61 (t), $J$ = 1.8 Hz] that the fourth new compound (**4**), $C_{10}H_{14}O_3$, is an $\alpha,\beta$-unsaturated $\gamma$-lactone. **4** also contains three methyl groups of which two are attached to a fully substituted oxygen-carrying carbon atom ($^1H$ NMR, a six-proton singlet at $\delta$ 1.48; $^{13}C$ NMR signal at $\delta$ 87.4; IR bands at 1375 and 1365 $cm^{-1}$) and one is part of a methyl ketone group (IR band at 1725 $cm^{-1}$; $^1H$ NMR, three-proton singlet at $\delta$ 2.24). A spin decoupling experiment was applied to demonstrate that the latter is present in a 3-oxobutyl moiety, which is linked to the fully substituted olefinic $\beta$-carbon atom ($^{13}C$ NMR $\delta$ 176.3). These results allowed the identification of **4** as 4-methyl-3-(3-oxo-1-butyl)-2-penten-4-olide.

The structure of **4** was confirmed by an HMBC experiment. Importantly, couplings were observed between the protons of the two methyl groups resonating at $\delta$ 1.48 (H-5, H-6) and C-4 as well as C-3. The olefinic proton (H-2) was correlated with C-1 and C-4, and the two protons at C-8 were correlated with C-3, C-7, and C-9.

Compound **5**, $C_9H_{12}O_3$, was formulated as 3-methyl-7-oxo-2-octen-4-olide with the aid of spectral results. Thus, the IR spectrum, containing absorption bands at 1760 and 1650 $cm^{-1}$, was consistent with the presence of the $\alpha,\beta$-unsaturated $\gamma$-lactone system. H-2, the $\alpha$-proton, gave rise to a quintet at $\delta$ 5.82, H-4 to a complex signal at $\delta$ 4.86,

**Scheme I. Important Fragmentation Reactions in the Mass Spectra of 5 and 6**




H-8 to a three-proton singlet at $\delta$ 2.18, and H-9 to a narrowly split doublet of doublets at $\delta$ 2.09 in the $^1$H NMR spectrum.

Correlations found in the HMBC spectrum were used to establish the connectivity between H-2 and the lactonic carbonyl carbon atom (C-1 at $\delta$ 172.1). The latter could not be detected in the $^{13}$C NMR spectrum due to low sample concentration. H-2 also showed a three-bond coupling to the lactonic methine carbon atom (C-4), while the protons of the vinylic methyl group (H-9) were correlated with C-2, C-3, and C-4. Correlations were also found between H-8 and C-6 and C-7 and between H-6 and C-5 and C-7.

The mass spectrum of **5** contains diagnostically useful peaks at *m/z* 168 (M), 111, 98, 97, 58, and 43. These may be ascribed to ions formed by the bond-breaking reactions outlined in Scheme I. It is noteworthy that abundant *m/z* 98 ions ($C_5H_6O_2$) are present in the mass spectra of the lactones 1 and **2** as well.

The sixth new compound (**6**), $C_{10}H_{12}O_3$, was identified as 4-(5-methyl-2-furyl)pentan-4-olide. Its IR spectrum displayed absorption bands at 1780 and 1560 cm$^{-1}$, consistent with the presence of the saturated $\gamma$-lactone group and the furan group. The olefinic protons, H-7 and H-8, are vicinally coupled and appeared as a doublet at $\delta$ 6.20 ($J$ = 3.2 Hz) and a doublet of quartets at $\delta$ 5.90 ($J$ = 1.0 and 3.2 Hz), respectively. Of the methyl groups, H-5, being attached to the fully substituted C-4, gave rise to a singlet at $\delta$ 1.76, while H-10, being coupled to H-8, resonated as a narrowly split doublet at $\delta$ 2.28 in the $^1$H NMR spectrum.

Useful structural information was also provided by the mass spectrum of **6**. This includes intense peaks at *m/z* 180 (M), 165, 136, 125, 121, and 109, which are proposed to correspond to ions arising as summarized in Scheme I.

Attempts to synthesize **6** have been unsuccessful. Treatment of 2-[5-methyl-5-(5-methyl-2-furyl)tetrahydro-2-furyl]-2-propanol (**26**) (Almqvist et al., 1974) with pyridinium chlorochromate in dichloromethane, however, afforded a mixture of products. One of these gave rise to a mass spectrum identical with that of **6**.

The seventh new compound (**7**), $C_{11}H_{16}O_3$, possesses a saturated $\gamma$-lactone moiety (IR band at 1791 cm$^{-1}$; $^{13}$C NMR signals at $\delta$ 160.8 and 80.2) and an ether oxygen linked to two methine carbon atoms ($^{13}$C NMR signals at $\delta$ 77.6 and 78.5; no OH absorption in the IR spectrum). The $^1$H NMR spectrum was consistent with the presence of two methyl groups, of which one, giving rise to a singlet at $\delta$ 1.32, is attached to a fully substituted oxygen-carrying carbon atom and the other resonating at $\delta$ 1.73 is part of an isopropenyl group ($^{13}$C NMR $\delta$ 18.5, 111.1, and 145.2). Since the $^{13}$C NMR spectrum was devoid of signals due to additional sp$^2$ carbon atoms, it followed that **7** has a bicyclic structure.

The structural fragments identified were allocated to a 3,7-epoxy-4,8-dimethyl-8-nonen-4-olide structure with the aid of correlations found in the COSY and HMBC spectra. The presence of cross-peaks due to two- and three-bond couplings between the methyl group protons at $\delta$ 1.32 (H-11) and C-3, C-4, and C-5 were of particular importance, as was the fact that H-9 and H-10 of the isopropenyl group showed long-range coupling to C-7.

Stereochemical information was provided by NOE difference experiments. These involved irradiation at the frequency of the signal due to H-3 and resulted in the enhancement of the signals due to H-2b, H-7, and H-11. Conversely, irradiation at the frequency of the signal due to H-11 gave rise to NOE enhancements on the signals due to H-2b and H-3. H-3, H-7, and H-11 are hence situated on the same face of the molecule; i.e., the relative stereochemistry of **7** is 3*R**,4*R**,7*R**. Inspection of Dreiding models then shows that if **7** exists in a twist-boat conformation, the torsion angle between the pro-*S* hydrogen at C-2 (H-2a) and the hydrogen at C-3 is close to 90°, which is consistent with the observed absence of vicinal coupling between these protons.

The eighth new compound (**8**), $C_8H_{10}O_2$, was formulated as 3-isopropyl-2,4-pentadien-5-olide. Its IR spectrum, containing absorption bands at 1742, 1640, and 1553 cm$^{-1}$, is consistent with the presence of the $\alpha$-pyrone system. The protons of the isopropyl substituent, H-6–H-8, appeared as a doublet of septets at $\delta$ 1.85 ($J$ = 1.0 and 6.8 Hz) and a six-proton doublet at $\delta$ 0.62 ($C_6D_6$) in the $^1$H NMR spectrum. H-2, which is long-range-coupled to H-4, H-5, and H-6, resonated as a doublet of triplets at $\delta$ 5.83, while H-4 and H-5 ($\delta$ 5.11 and 6.53, respectively) are vicinally coupled, the magnitude of the coupling constant, $J$ = 5.5 Hz, being diagnostic of the substitution pattern in **8**.

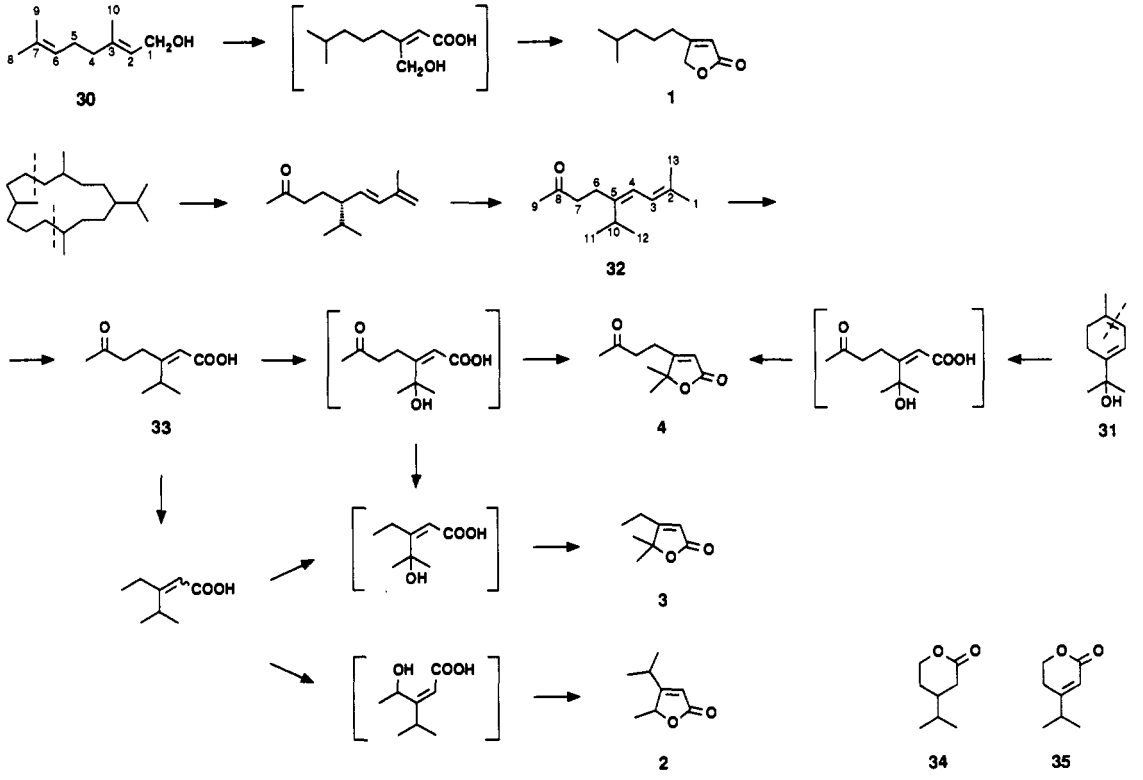
It was concluded from the spectral data that the ninth new compound (**9**), $C_{13}H_{22}O_2$, contains three methyl groups, which are attached to fully substituted sp$^3$ carbon atoms. The two oxygen atoms are present in a lactone moiety [IR band at 1735 cm$^{-1}$; $^{13}$C NMR signals at $\delta$ 64.8 (t) and 176.6 (s)]. Since the $^1$H and $^{13}$C NMR spectra were devoid of signals due to double bonds, it followed that **9** has a bicyclic structure. These data in conjunction with results from the COSY and HMQC spectra preliminarily identified **9** as a 4-oxa-7,11,11-trimethylbicyclo-[5.4.0]undecan-3-one. W-couplings were observed in the COSY spectrum between the methyl group at the ring junction (H-14 at $\delta$ 1.08) and H-6b and H-8a. In addition, H-10a was W-coupled to H-13, i.e., the axial methyl group attached to C-11. The NOESY spectrum included cross-peaks due to interactions between H-14 and H-2b, H-5b, and H-13, and between H-2a and H-12 and H-13. These results established the carbon–carbon connectivity and suggested that the stereochemistry at the ring junction is *trans*.

A synthesis was performed to confirm this assignment and to determine the absolute configuration of **9**. This involved a Baeyer–Villiger oxidation of (1*S*,6*R*)-6,10,10-trimethylbicyclo[4.4.0]decan-3-one (**27**), prepared according to the method of Jansen et al. (1989) from (−)-dihydrocarvone (**29**). The major product, (1*S*,7*R*)-4-oxa-7,11,11-trimethylbicyclo[5.4.0]undecan-3-one, proved to be identical in all respects with the naturally occurring **9**.

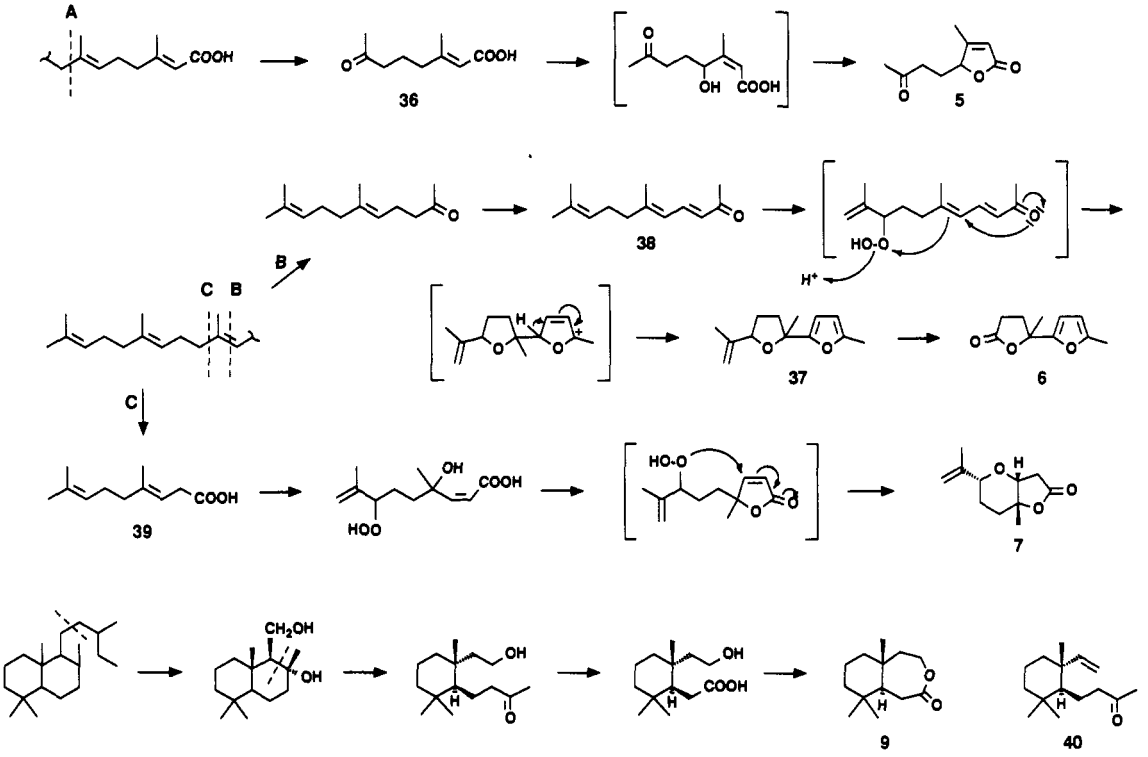
Compound **10** gave rise to IR, $^1$H NMR, and mass spectra identical with those of (*Z*)-6-nonen-4-olide, a $\gamma$-lactone previously isolated from tuberose absolute (*Polianthes tuberosa* L.) by Maurer and Hauser (1982). Racemic (*Z*)-6-nonen-4-olide has been synthesized (Alexakis et al., 1980).

5-Methyl-4-hexanolide (**11**), now isolated from tobacco, has previously been reported as a constituent of lavender oil (*Lavandula vera* D.C.) (Timmer et al., 1975). This

**Scheme II. Proposed Biogenesis of 1–4: Formulas 34 and 35**



**Scheme III. Proposed Biogenesis of 5–7 and 9: Formula 40**



compound has also been prepared by synthesis (Grimm and Reissig, 1985; Gassman and De Silva, 1991).

Compounds 12 and 13 were identified as (3*R*,4*R*)- and (3*R*,4*S*)-3-methyl-4-pentanolide by comparison of their optical rotation and spectral data with those published for corresponding synthetic samples (Byström et al., 1981; Najera et al., 1984; Mosandl and Gunther, 1989). These lactones are probably identical with the two isomeric 3-methyl-4-pentanolides previously reported as tobacco constituents (Roberts and Rohde, 1972).

## DISCUSSION

Of the newly isolated tobacco lactones, 1 is evidently a monoterpenoid. As outlined in Scheme II, its formation may be explained by oxidation at C-1 and C-10 and selective hydrogenation of the 6,7 double bond in geraniol (30). Support for this view is provided by the fact that both geraniol (30) and the corresponding geranic acid are tobacco constituents (Demole and Berthet, 1972a,b).

Lactone 4 may also be of monoterpenoid origin and

generated via oxidative breakage of the 1,2 double bond in a precursor such as *p*-mentha-1,3-dien-8-ol (**31**). It may well be, however, that isosolanone (**32**), which is a degraded cembranoid and a well-known tobacco constituent, can serve as an alternative precursor (Demole and Berthet, 1972a). Oxidative rupture of the 2,3 double bond to form the oxoacid **33** and subsequent hydroxylation would then be involved in the formation of lactone **4**.

The oxoacid **33**, which has been found in tobacco (Chuman and Noguchi, 1975; Kimland et al., 1973), may also be an intermediate in the biogenesis of lactones **2** and **3**. A formal loss of two carbon atoms and allylic oxidation are the prerequisite steps.

Lactone **8** may also be classified as a degraded cembranoid. This compound is structurally closely related to the lactones **34** and **35**, both of which are tobacco constituents (Kaneko and Harada, 1972; Roberts and Rohde, 1972; Demole and Berthet, 1972a).

The biogenesis of lactones **5**–**7** may be envisaged to take place via degradation of carboacyclic isoprenoid precursors. Thus, as illustrated in Scheme III, the intermediary $C_9$ oxoacid **36** may arise via breakage A in an isoprenoid acid. Double-bond isomerization and allylic oxidation complete the route to **5**.

Lactone **6** is likely to derive from **37**, which is a tobacco constituent (Almqvist et al., 1974). The latter, in turn, has been suggested to arise via rupture B in an isoprenoid precursor, pseudoionone (**38**) being an intermediate. The formation of lactone **7** may be explained by a rupture of type C taking place in the isoprenoid precursor. The $C_{11}$ acid **39** obtained undergoes oxygenation, lactonization, and cyclization. It is noteworthy that the lactones **5** and **7** are both racemic; each was shown by GC on a Cyclodex B column to be a 1:1 mixture of enantiomers. This result implies that the oxygenation reactions invoked are not enantioselective. It should also be mentioned that the tobacco lactones (6*Z*)-nonen-4-olide (**10**), 2-nonen-4-olide (Weeks et al., 1989), and 4-nonanolide (Schumacher and Vestal, 1974) are present as mixtures of enantiomers. It is also noteworthy that the isomeric 3-methyl-4-pentanolides now isolated from Greek tobacco (**12** and **13**) are enantiomerically homogeneous and differ with respect to the configuration at C-4. These results are in variance with previous findings that 4-alkyl-substituted $\gamma$-lactones in, e.g., strawberries are enantiomerically homogeneous (Mosandl et al., 1990).

Lactone **9** may be a degraded labdanoid arising as shown in Scheme III. This view is supported by the fact that **9** has the prerequisite absolute configuration and that the structurally related **40** is an experimentally verified degraded tobacco labdanoid (Hlubucek et al., 1974).

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