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Tobacco Chemistry. 82. Seven New Cembrane-Derived Compounds from Tobacco†

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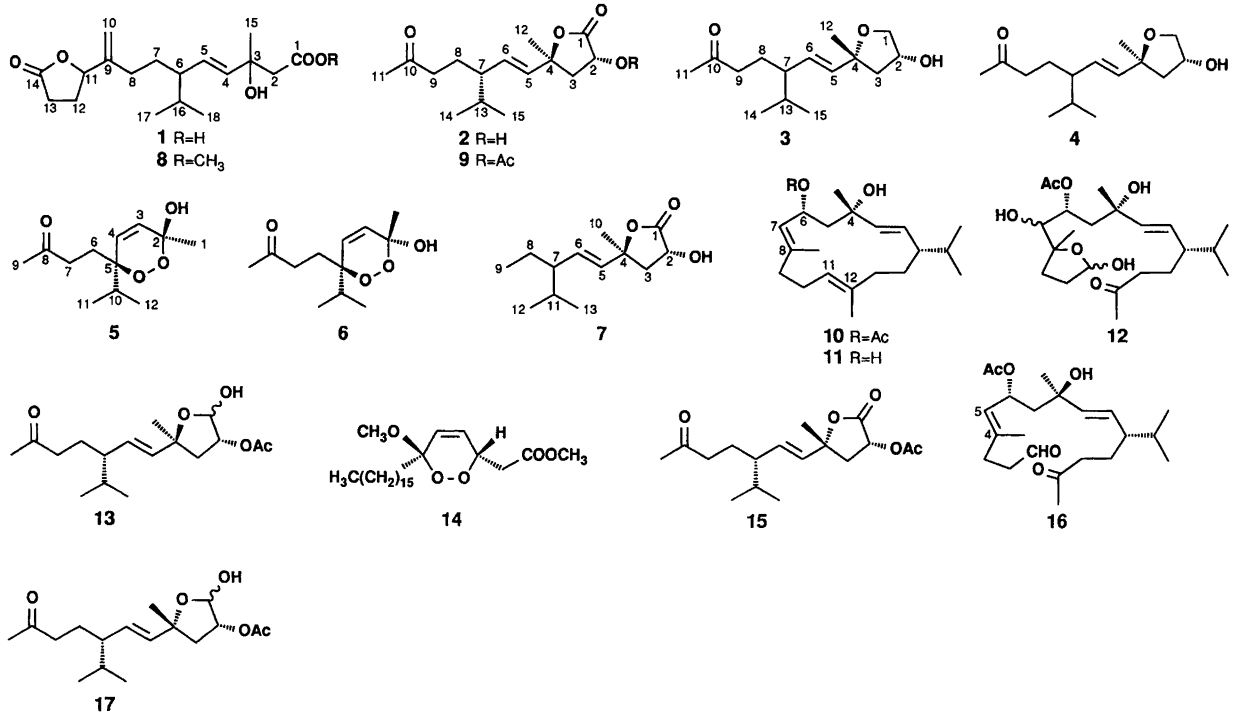
Seven new cembrane-derived compounds (**1**–**7**) have been isolated from tobacco. They have been identified as (4*E*)-3-hydroxy-6-isopropyl-3-methyl-9-(5-oxotetrahydrofuran-2-yl)-4,9-decadienoic acid (**1**), (2*R*,4*S*,5*E*,7*S*)-2-hydroxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (**2**), the (2*R**,4*R**,5*E*)- and (2*R**,4*S**,5*E*)-1,4-epoxy-2-hydroxy-7-isopropyl-4-methyl-5-undecen-10-ones **3** and **4**, the (2*S**,5*R**)- and (2*R**,5*R**)-2,5-epidioxy-2-hydroxy-5-isopropyl-3-nonen-8-ones **5** and **6** and (2*R**,4*S**,5*E*)-2-hydroxy-7-isopropyl-4-methyl-5-nonen-4-olide (**7**) by spectral methods and, in the case of **2**, also by biomimetic synthesis. The biogenesis of the new compounds is discussed.

The aroma fractions derived from tobacco are unusually rich sources of degraded isoprenoids. Among these is a group comprising more than seventy carboacyclic isopropyl-containing compounds. These compounds, which are unique to tobacco, are postulated to arise by biodegradation of the cembranic diterpenoids present in the cuticular wax of the leaf and flower.[2,3] They make important contributions to the aroma of tobacco. We now report the isolation by HPLC of seven new cembrane-derived compounds (**1**–**7**) from tobacco.



† For part 81 see Ref. 1.
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Results

Structure elucidation. The first new compound **1**, (4*E*)-3-hydroxy-6-isopropyl-3-methyl-9-(5-oxotetrahydrofuran-2-yl)-4,9-decadienoic acid, was isolated as the corresponding methyl ester (**8**) from the acidic fraction of a chloroform extract of flowers of Greek tobacco. The IR spectrum of **8**, $C_{19}H_{30}O_5$, contained bands at 3525, 3090, 1785 and 1723 cm^{-1} consistent with the presence of the hydroxy group, the 1,1-disubstituted double bond, the γ-lactone moiety and the methyl ester group. The ^1H NMR spectrum displayed the signals due to H-17 and H-18 of the isopropyl group as three-proton doublets at δ 0.82 and 0.87 and those due to H-15 and the methoxycarbonyl group as three-proton singlets at δ 1.32 and 3.67, respectively. H-11, the lactonic hydrogen, appeared as a triplet at δ 4.89, H-10a and H-10b of the 1,1-disubstituted double bond as broad singlets at δ 4.93 and 5.10 and H-4 and H-5 of the 1,2-disubstituted double bond as a doublet and a doublet of doublets ($J_{4,5}$ 15.5 Hz) at δ 5.49 and 5.41, respectively.

The 2D-NMR spectra, particularly the HMBC spectrum, included a wealth of structural information. Thus, as shown in Table 1, correlations were observed between, *inter alia*, H-15 and C-2; H-2ab and C-4; the hydroxy hydrogen and C-3; H-16 and C-6; H-8ab and C-10; and H-11 and C-10. While these were used to formulate the gross structures of **1** and **8**, the relative stereochemistry and absolute configuration remained to be resolved.

The second new compound (**2**), $C_{15}H_{24}O_4$, was also isolated from the extract of flowers of Greek tobacco. **2** contains a γ-lactone moiety and a methyl ketone group [IR bands at 1786 and 1719 cm^{-1}; ^1H NMR: methyl singlet at δ 2.12; ^{13}C NMR signals at δ 82.7 (s), 176.8 (s) and 208.7 (s)]. The remaining oxygen atom is present as a secondary hydroxy group (IR band at 3408 cm^{-1}; ^1H NMR: doublet of doublets at δ 4.50 shifted to δ 5.57 in the spectrum of the monoacetate **9**).

The ^1H NMR spectrum of lactone **2** was also consistent with the presence of an isopropyl group, a methyl group linked to a fully substituted oxygen-carrying carbon atom (three-proton doublets at δ 0.81 and 0.87 and a three-proton singlet at δ 1.56) and a 1,2-disubstituted double bond of *E*-geometry (doublet of doublets at δ 5.42 and a doublet at δ 5.47, $J_{1,2}$ 15.6 Hz). These structural fragments were shown to be linked into a 2-hydroxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide structure with the use of the HMBC spectrum. Of

Table 1. HMBC[a] or COSY[b] correlations for compounds **2**, **3**, **5**, **7** and **8**[c]

	Compound				
Proton	**2**[a]	**3**[a]	**5**[a]	**7**[b]	**8**[a]
H-1		C-2, C-3, C-4	C-2, C-3		
H-2	C-1, C-3			H-3	C-1, C-3, C-4, C-15
H-3	C-1, C-2, C-4, C-5, C-12	C-1, C-4, C-5, C-12	C-2, C-5	H-2, H-3′	
H-4			C-2, C-5		C-3, C-5, C-6
H-5	C-4, C-6, C-7	C-4, C-6, C-7, C-12		H-6	C-3, C-4, C-6
H-6	C-4, C-5, C-7	C-4, C-5	C-4, C-5, C-7, C-8	H-5, H-7	C-8
H-7	C-6		C-6, C-8	H-6, H-8	C-5, C-6, C-8
H-8				H-7, H-8′, H-9	C-6, C-7, C-9, C-10, C-11
H-9	C-7, C-8, C-10, C-11	C-7, C-8, C-10	C-8	H-8	
H-10			C-5, C-11, C-12		C-8, C-9, C-11
H-11	C-10	C-10	C-5, C-10, C-12	H-12, H-13	C-10
H-12	C-3, C-4, C-5	C-3, C-4, C-5	C-5, C-10, C-11	H-11	C-9, C-11, C-13, C-14
H-13	C-14, C-15	C-6, C-7, C-8, C-14, C-15		H-11	C-11, C-12, C-14
H-14	C-7, C-13, C-15	C-7, C-13, C-15			
H-15	C-7, C-13, C-14	C-7, C-13, C-14			C-2, C-3, C-4
H-16					C-5, C-6, C-7, C-17, C-18
H-17					C-6, C-16, C-18
H-18					C-6, C-16, C-17
–OH			C-1, C-2		C-3, C-15
$-OCH_3$					C-1

[c] The carbon resonances were assigned by means of an HMQC experiment.

particular diagnostic importance were the correlations between H-2 and C-1; H-12 and C-3 and C-4; H-5 and C-4; H-14/H-15 and C-7; H-9ab and C-10; and H-11 and C-10 (Table 1). The stereochemistry and the absolute configuration of **2** were then determined by a biomimetic synthesis, the 6-acetate (**10**) of the major tobacco cembranoid, the (4*S*,6*R*)-diol **11**,[4,5] being used as the starting material.

The prerequisite breakages of the 7,8 and 11,12 double bonds were accomplished with the aid of sodium periodate and a catalytic amount of osmium tetraoxide. Preliminary data suggest that the C_{20} hemiacetals **12** formed via an initial rupture of the 11,12 double bond and dihydroxylation of the 7,8 double bond are intermediates and that these, in turn, undergo breakage of the 7,8 bond to give the C_{15} hemiacetals **13**. Subsequent oxidation by using pyridinium dichromate (PDC) in dimethylformamide afforded the desired (2*R*,4*S*,5*E*,7*S*)-2-acetoxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide. This compound proved to be identical with the acetate (**9**) derived from the naturally occurring **2**, hence lending support to the view that the latter is biogenetically closely related to the tobacco cembranoids.

The third and fourth new compound (**3**, **4**; $C_{15}H_{26}O_3$) were isolated from a CH_2Cl_2 extract of moist snuff, but were also found to be present by GC–MS in a diethyl ether extract of sun-cured leaves of Greek tobacco. They were identified as isomers of (5*E*)-1,4-epoxy-2-hydroxy-7-isopropyl-4-methyl-5-undecen-10-one by using spectral methods. Thus, H-11 being adjacent to the oxo group at C-10 and H-12 to the oxygen-carrying C-4 resonated as three-proton singlets at δ 2.11 and 1.31 for **3** and at δ 2.12 and 1.44 for **4**.

The presence of the 1,4-epoxy-2-hydroxy system was established by spin simulation experiments involving the H-1 to H-3 portion of the molecule. In addition, the COSY spectra included correlations between H-2 (resonating as a multiplet at δ 4.44 and 4.48 for **3** and **4**, respectively) and the protons attached to C-1 and C-3. Evidence was also provided by the HMBC spectra, in which correlations were observed between the protons at C-1 (present at δ 3.82 and 4.06 for **3** and at δ 3.80 and 3.88 for **4**) and C-2 and C-4 and between the protons at C-3 and C-1, C-4 and C-12. Moreover, the correlation between the protons at C-3 and the olefinic C-5 was used to assign the *E*-1,2-disubstituted double bond to the 5,6-position. Similarly, the assignment of the isopropyl substituent to C-7 rested on the observed correlation between this carbon atom and H-14 and H-15 of the isopropyl group (Table 1).

Information on the relative stereochemistry at C-2 and C-4 in **3** and **4** was obtained from NOE difference spectroscopy. Thus, in the case of **3** both H-2 and H-12 showed interaction with H-1b and H-3a, which is consonant with a *cis*-relationship between H-2 and H-12, i.e., a 2*R**,4*R**-stereochemistry. The NOE difference spectra of **4** showed responses between H-2 and H-1b and H-3b on the one hand and between H-12 and H-3a, H-5 and H-6 on the other. These results indicated that H-2 and H-12 are *trans*, i.e., the relative stereochemistry is 2*R**,4*S**. The chirality of C-7 in **3** and **4** remains to be determined.

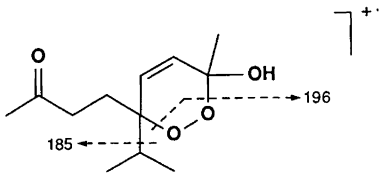
The fifth new compound (**5**, $C_{12}H_{20}O_4$), which was obtained from a diethyl ether extract of sun-cured leaves of Greek tobacco, was identified as a 2,5-epidioxy-2-hydroxy-5-isopropyl-3-nonen-8-one by analysis of the spectral data.

H-11 and H-12, which form part of the isopropyl group appear as doublets at δ 0.95 and 1.00 in the ^{1}H NMR spectrum, while H-10 resonates as a septet at δ 2.25. The remaining two methyl groups, H-1 and H-9, which appear at δ 1.38 and 2.15, are linked to the fully substituted oxygen-carrying C-2 and the carbonyl carbon C-8, respectively. H-3 and H-4 of the *Z*-disubstituted double bond (*J* 10.3 Hz) give rise to doublets at δ 5.95 and 5.87, respectively. C-2 and C-5, both being linked to the epidioxy group and C-2 also to the hydroxy group, appear at δ 95.1 and 83.4 in the ^{13}C NMR spectrum.

The HMBC spectrum was very informative (see Table 1). Particularly useful for the determination of the carbon–carbon connectivity were the correlations between the hydroxy hydrogen (resonating at δ 3.62) and C-1 and C-2, between H-3 and C-5, between H-11/H-12 and C-5, between H-6b and C-4 and between H-6a and C-8.

The mass spectrum was also diagnostically useful. The peak at the highest *m/z* in the electron impact spectrum is present at mass 196. It corresponds to a $C_{12}H_{20}O_2$ ion formed by loss of O_2 from the molecular ion (see Scheme 1). An analogous loss of 32 amu is observed in the spectra of other epidioxides, e.g., chondrillin (**14**).[6] The peak at *m/z* 185 is due to a $C_9H_{13}O_4$ ion generated by loss of a propyl radical through α-cleavage. The chemical ionization mass spectrum obtained by using ammonia as the reagent gas displayed the $[M + NH_4]^+$ ion at mass 246.

A comparison of the spectral characteristics suggested that the sixth new compound (**6**, $C_{12}H_{20}O_4$) is a 2,5-epidioxy-2-hydroxy-5-isopropyl-3-nonen-8-one isomeric with **5**. The differences observed, e.g., the chemical shift values of C-6 and C-10 (24.7 and 33.5 for **5** as against 27.2 and 31.2 for **6**) are explained by **5** and **6** differing with respect to the relative stereochemistry at C-2 and C-5. This conclusion was supported by NOE measurements involving H-1, the methyl group attached to the hydroxy-bearing C-2, and H-10 of the isopropyl group. A response was



5, 6

Scheme 1. Important fragmentation reactions in the mass spectra of compounds **5** and **6**.

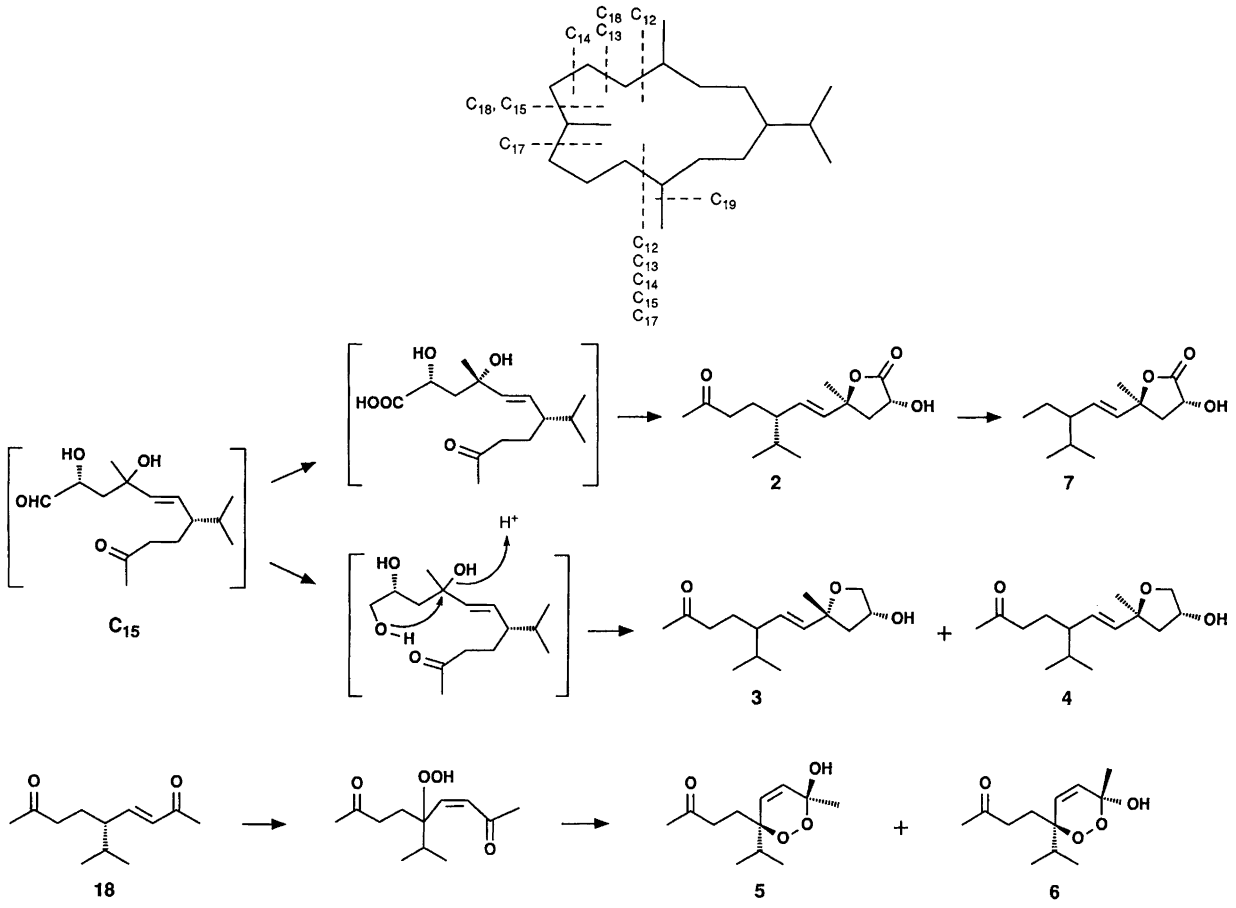
registered for **5** but not for **6** indicating that these two groups are *cis* in **5** and *trans* in **6**, i.e., the relative stereochemistry is (2*S**,5*R**) and (2*R**,5*R**), respectively.

The seventh new compound (**7**, $C_{13}H_{22}O_3$) was also isolated from the extract of leaves of Greek tobacco. Its ^{1}H NMR spectrum demonstrated the presence of four methyl groups, of which one is present in an ethyl group, two form part of an isopropyl group and one is attached to a fully substituted oxygen-carrying carbon atom [three-proton signals at δ 0.78 (t), 0.80 (d), 0.85 (d) and 1.56 (s)]. Compound **7** also contains a double bond which is *E*-disubstituted (J = 15.6 Hz) and gives rise to a doublet and a doublet of doublets at δ 4.99 and 5.23, respectively, in the ^{1}H NMR spectrum (C_6D_6).

The oxygen atoms in **7** are accounted for by a secondary hydroxy group and a γ-lactone group [IR bands at 3574, 3404 and 1790 cm^{-1}; ^{13}C NMR signals at δ 68.6 (d), 82.8 (s) and 177.0 (s)]. The DQ COSY spectrum was then used to piece together the structural fragments identified into a 2-hydroxy-7-isopropyl-4-methyl-5*E*-nonen-4-olide structure (Table 1). Support for this assignment and particularly for the assignment of the hydroxy group to the position α to the lactonic oxo group was provided by a comparison of relevant ^{1}H and ^{13}C NMR data for **2** and **7**.

NOE difference spectroscopy was applied to study the relative stereochemistry of **7**. Effects were found between H-2 and H-3b and between H-10 and H-3a and H-5 suggesting that H-2 and H-10 are *trans*. Consistent with this assignment is the observation of NOEs between H-2 and H-3b and between H-12 and H-3a in **2** and in the acetate **9**, where a *trans*-relationship between H-2 and H-12 was firmly established by chemical means. In addition, the 4*R*-isomer (**15**) of the acetate **9** showed NOEs between H-2 and H-12 in keeping with the *cis*-relationship between these two protons. This isomer was prepared, for reference purposes, from the aldehyde **16**, a *seco*-cembranoid,[7] via breakage of the 4,5 double bond using sodium periodate and osmium tetraoxide followed by oxidation of the mixture of hemiacetals (**17**) formed with the use of PDC. Hence, it can be concluded that the naturally occurring **7** has a 2*R**,4*S**-stereochemistry, the chirality of C-7 being left to be determined.

Biogenesis. Although evidence from tracer experiments is not at hand, it is generally accepted that the large group of isopropyl-containing irregular isoprenoids present in tobacco is of cembranoid origin. The biodegradation of appropriate cembranic precursors has been suggested to involve initial breakages of the carbon–carbon bonds indicated in Scheme 2. The key metabolites generated



Scheme 2. Proposed biogenesis of compounds **2**–**7**.

have 19, 18, 17, 15, 14, 13 or 12 carbon atoms. They undergo further chemical transformations, which may involve loss of carbon atoms.[2]

Although undoubtedly of cembranic origin, the formation of the new C_{18} compound (**1**) is not explained by using this simplified model. At present, however, we do not have an insight into the fragmentation reactions taking place, which formally comprise loss of C-7 and C-19 from a parent cembranoid.

By contrast, the biogenesis of the new C_{15} compounds (**2–4**) is readily accounted for by oxidation or reduction followed by cyclization taking place with the C_{15} key metabolite. The C_{15} lactone **2** may, in turn, serve as an intermediate in the generation of the new C_{13} constituent **7**.

The new C_{12} constituents (**5**, **6**), which are the first degraded cembranoids containing an epidioxide moiety found in tobacco, are most likely derived from norsolanadione (**18**),[8] the C_{12} key metabolite. Introduction of a hydroperoxide group, double bond isomerization and formation of a hemiacetal are the necessary steps.

Experimental

High performance liquid chromatography was carried out using a Waters 6000A or a Delta Prep 3000 solvent delivery system, 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 25 Stereo DS 55 SM/DS 55 S mass spectrometer–computer system.

Isolation. (4*E*)-3-Hydroxy-6-isopropyl-3-methyl-9-(5-oxotetrahydrofuran-2-yl)-4,9-decadienoic acid (**1**) was isolated as the corresponding methyl ester (**8**, 5.1 mg) from fraction 2 (64 mg) of the acidic portion of a $CHCl_3$ extract of flowers of Greek tobacco[7] by HPLC using a column packed with Spherisorb 5 Nitrile (hexane–EtOAc 60:40). (2*R*,4*S*,5*E*,7*S*)-2-Hydroxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (**2**, 4.6 mg) was obtained from fraction C43 (4.2 g) of a $CHCl_3$ extract of flowers of Greek tobacco[9] by repetitive HPLC using columns packed with Spherisorb 5 ODS (MeOH–H_2O 90:10), Lichrosorb Diol (hexane–EtOAc 40:60) and Spherisorb 5 ODS (MeOH–H_2O 80:20). (2*R**,4*R**,5*E*)-1,4-Epoxy-2-hydroxy-7-isopropyl-4-methyl-5-undecen-10-one (**3**, 10.3 mg) and the corresponding 4*S**-epimer (**4**, 7.0 mg) were isolated from a CH_2Cl_2 extract (20 g) of moist snuff tobacco by flash chromatography over silica gel using a gradient of hexane–EtOAc–MeOH as the eluent followed by HPLC of fraction 6 (1.57 g; out of fractions 1–9) using columns packed with Spherisorb 5 (hexane–EtOAc 30:70) and Spherisorb 5 Nitrile (hexane–EtOAc 70:30). (2*S**,5*R**)-2,5-Epidioxy-2-hydroxy-5-isopropyl-3-nonen-8-one (**5**, 12.9 mg) and the corresponding 2*R**,5*R**-isomer **6** (4.8 mg) were isolated from fraction B8 and (2*R**,4*S**,5*E*)-2-hydroxy-7-isopropyl-4-methyl-5-nonen-4-olide (**7**, 1.4 mg) from fraction B7 of an Et_2O extract of 295 kg of sun-cured Greek tobacco[10] by chromatography over silica gel using hexane–EtOAc as the eluent followed by HPLC using columns packed with Spherisorb 5 Nitrile (hexane–EtOAc 80:20), Spherisorb 5 (hexane–EtOAc 60:40) and Lichrosorb Diol (hexane–EtOAc 80:20).

(4*E*)-Methyl 3-hydroxy-6-isopropyl-3-methyl-9-(5-oxotetrahydrofuran-2-yl)-4,9-decadienoate (**8**) was an oil and had $[\alpha]_D$ −4.4° (*c* 0.45, $CHCl_3$); (Found: $[M-15]^+$ 323.1923. Calc. for $C_{18}H_{27}O_5$: 323.1858); IR (CCl_4): 3525, 3090, 1785, 1723, 1384, 1368, 1178, 981 and 909 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, *J* 6.9 Hz)/0.87 (d, *J* 6.7 Hz) (H-17/H-18), 1.32 (s, H-15), 2.55 (m, H-13a and H-13b), 2.58 (m, H-2a and H-2b), 3.67 (s, $-OCH_3$), 3.87 (s, $-OH$), 4.89 (t, *J* 7.5 Hz, H-11), 4.93 (br s, H-10a), 5.10 (br s, H-10b), 5.41 (dd, *J* 8.5 and 15.5 Hz, H-5) and 5.49 (d, *J* 15.5 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 18.9/20.7 (C-17/C-18), 27.4 (C-12), 28.5 (C-13), 28.8 (C-15), 29.3 (C-8), 30.3 (C-7), 32.0 (C-16), 45.4 (C-2), 48.5 (C-6), 51.7 ($-OCH_3$), 71.0 (C-3), 81.9 (C-11), 110.6 (C-10), 130.1 (C-5), 137.2 (C-4), 146.5 (C-9), 173.0 (C-1) and 177.0 (C-14); MS [*m/z* (%, composition)]: 323 (4, $M-15$), 291 (8, $C_{17}H_{23}O_4$), 265 (21, $C_{16}H_{25}O_3$), 247 (5, $C_{16}H_{23}O_2$), 221 (7, $C_{17}H_{17}$), 203 (6, $C_{13}H_{15}O_2$), 179 (11, $C_{11}H_{15}O_2$), 161 (13, $C_{11}H_{13}O$), 147 (16), 135 (15, $C_{10}H_{15}$), 121 (26), 107 (19, C_8H_{11} and C_7H_7O), 97 (35, C_6H_9O and $C_5H_5O_2$), 81 (25, C_6H_9 and C_5H_5O), 69 (27), 55 (41) and 43 (100).

(2*R*,4*S*,5*E*,7*S*)-2-Hydroxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (**2**) was an oil and had $[\alpha]_D$ +18° (*c* 0.41, $CHCl_3$); (Found: M^+ 268.1687. Calc. for $C_{15}H_{24}O_4$: 268.1675); IR (CCl_4): 3408, 1786, 1719, 1386, 1368 and 984 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (d, *J* 6.8 Hz)/0.87 (d, *J* 6.7 Hz) (H-14/H-15), 1.56 (s, H-12), 2.09 (dd, *J* 10.6 and −12.6 Hz, H-3a), 2.12 (s, H-11), 2.31 (m, H-9a and H-9b), 2.67 (dd, *J* 8.2 and −12.6 Hz, H-3b), 4.50 (dd, *J* 8.2 and 10.6 Hz, H-2), 5.42 (dd, *J* 8.0 and 15.6 Hz, H-6) and 5.47 (d, *J* 15.6 Hz, H-5); ^{13}C NMR ($CDCl_3$): δ 19.0/20.7 (C-14/C-15), 25.9 (C-8), 28.0 (C-12), 30.0 (C-11), 31.8 (C-13), 41.8 (C-9), 42.3 (C-3), 48.5 (C-7), 68.6 (C-2), 82.7 (C-4), 131.9 (C-6), 133.2 (C-5), 176.8 (C-1) and 208.7 (C-10); MS [*m/z* (%, composition)]: 268 (0.2, *M*), 250 (0.3, $C_{15}H_{22}O_3$), 225 (0.6, $C_{12}H_{17}O_4$ and $C_{13}H_{21}O_3$), 194 (7, $C_{13}H_{22}O$), 175 (4), 136 (29, $C_{10}H_{16}$ and $C_9H_{12}O$), 121 (32, C_9H_{13} and C_8H_9O), 109 (14, C_8H_{13} and C_7H_9O), 93 (73, C_7H_9), 79 (21, C_6H_7), 69 (17, C_5H_9), 55 (19, C_4H_7 and C_3H_3O) and 43 (100, C_2H_3O and C_3H_7).

(2*R**,4*R**,5*E*)-1,4-Epoxy-2-hydroxy-7-isopropyl-4-methyl-5-undecen-10-one (**3**) was an oil and had $[\alpha]_D$ −7.8° (*c* 0.86, $CHCl_3$); (Found: M^+ 254.1906. Calc. for $C_{15}H_{26}O_3$: 254.1882); IR (CCl_4): 3627, 3571, 3480, 1719 and 1369 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* 6.8 Hz)/0.88 (d, *J* 6.7 Hz) (H-14/H-15), 1.31 (s, H-12), 1.99 (dd,

J 5.7 and −13.5 Hz, H-3a), 2.04 (ddd, *J* 1.0, 2.4 and −13.5 Hz, H-3b), 2.11 (s, H-11), 2.37 (m, H-9a and H-9b), 3.82 (ddd, *J* 1.0, 2.4 and −9.8 Hz, H-1a), 4.06 (dd, *J* 4.7 and −9.8 Hz, H-1b), 4.44 (m, H-2), 5.46 (dd, *J* 9.1 and 15.6 Hz, H-6) and 5.59 (d, *J* 15.6 Hz, H-5); ^{13}C NMR ($CDCl_3$): δ 19.3/20.9 (C-14/C-15), 26.3 (C-8), 27.9 (C-12), 30.2 (C-11), 32.3 (C-13), 42.1 (C-9), 47.5 (C-3), 48.6 (C-7), 73.8 (C-2), 75.2 (C-1), 82.2 (C-4), 129.3 (C-6), 138.3 (C-5) and 209.2 (C-10); MS [*m/z* (%, composition)]: 254 (0.4, *M*), 239 (0.5, $C_{14}H_{23}O_3$), 236 (0.4, $C_{15}H_{24}O_2$), 221 (0.3, $C_{14}H_{21}O_2$), 211 (0.5, $C_{12}H_{19}O_3$), 194 (13, $C_{13}H_{22}O$ and $C_{12}H_{18}O_2$), 181 (3, $C_{11}H_{17}O_2$), 153 (8, $C_9H_{13}O_2$ and $C_{10}H_{17}O$), 136 (22, $C_{10}H_{16}$), 127 (8, $C_7H_{11}O_2$), 121 (11, C_9H_{13}), 113 (11, $C_6H_9O_2$), 101 (20, $C_5H_9O_2$), 95 (13), 93 (22, C_7H_9), 81 (12, C_6H_9 and C_5H_5O), 69 (9, C_5H_9 and C_4H_5O), 55 (12, C_4H_7 and C_3H_3O) and 43 (100).

(2*R**,4*S**,5*E*)-1,4-Epoxy-2-hydroxy-7-isopropyl-4-methyl-5-undecen-10-one (**4**) was an oil and had $[\alpha]_D$ +12° (*c* 0.67, $CHCl_3$); (Found: $M^{+\cdot}$ 254.1892. Calc. for $C_{15}H_{26}O_3$: 254.1882); IR (CCl_4): 3628, 3447, 1720, 1368 and 981 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.81 (d, *J* 6.7 Hz)/0.87 (d, *J* 6.6 Hz) (H-14/H-15), 1.44 (s, H-12), 1.79 (ddd, *J* 1.1, 3.3 and −13.4 Hz, H-3a), 2.12 (s, H-11), 2.23 (dd, *J* 6.6 and −13.4 Hz, H-3b), 2.34 (m, H-9a and H-9b), 3.80 (ddd, *J* 1.1, 2.6 and −9.8 Hz, H-1a), 3.88 (dd, *J* 4.7 and −9.8 Hz, H-1b), 4.48 (m, H-2), 5.27 (dd, *J* 9.0 and 15.6 Hz, H-6) and 5.40 (d, *J* 15.6 Hz, H-5); ^{13}C NMR ($CDCl_3$): δ 19.0/20.7 (C-14/C-15), 26.2 (C-8), 27.9 (C-12), 30.1 (C-11), 32.0 (C-13), 42.1 (C-9), 47.0 (C-3), 48.6 (C-7), 73.4 (C-2), 74.4 (C-1), 82.4 (C-4), 129.0 (C-6), 137.1 (C-5) and 209.2 (C-10); MS [*m/z* (%, composition)]: 254 (0.5, *M*), 239 (4, $C_{14}H_{23}O_3$), 236 (0.4, $C_{15}H_{24}O_2$), 221 (1, $C_{14}H_{21}O_2$), 211 (1, $C_{12}H_{19}O_3$ and $C_{13}H_{23}O_2$), 194 (10, $C_{13}H_{22}O$ and $C_{12}H_{18}O_2$), 181 (4, $C_{11}H_{17}O_2$ and $C_{12}H_{21}O$), 153 (7, $C_9H_{13}O_2$ and $C_{10}H_{17}O$), 136 (15, $C_{10}H_{16}$), 127 (21, $C_7H_{11}O_2$), 121 (10, C_9H_{13} and C_8H_9O), 113 (23, $C_6H_9O_2$), 101 (14, $C_5H_9O_2$), 95 (13, C_6H_7O and C_7H_{11}), 93 (16, C_7H_9 and C_6H_5O), 81 (14, C_6H_9 and C_5H_5O), 69 (9, C_5H_9 and C_4H_5O), 55 (12, C_4H_7 and C_3H_3O) and 43 (100, C_2H_3O and C_3H_7).

(2*S**,5*R**)-2,5-Epidioxy-2-hydroxy-5-isopropyl-3-nonen-8-one (**5**) was an oil and had $[\alpha]_D$ +0.2° (*c* 1.2, $CHCl_3$); (Found: $[M-32]^{+\cdot}$ 196.1410. Calc. for $C_{12}H_{20}O_2$: 196.1463); IR (CCl_4): 3595, 3481, 1719, 1371, 1359, 1161, 1138, 996, 946 and 912 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.95 (d, *J* 7.0 Hz)/1.00 (d, *J* 7.0 Hz) (H-11/H-12), 1.38 (s, H-1), 1.83 (ddd, *J* 6.2, 7.1 and −15.0 Hz, H-6a), 1.93 (ddd, *J* 7.0, 8.3 and −15.0 Hz, H-6b), 2.15 (d, *J* 0.5 Hz, H-9), 2.25 (septet, *J* 7.0 Hz, H-10), 2.39 (m, H-7a and H-7b), 3.62 (br s, O*H*), 5.87 (d, *J* 10.3 Hz, H-4) and 5.95 (d, *J* 10.3 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 17.1/17.6 (C-11/C-12), 23.0 (C-1), 24.7 (C-6), 29.1 (C-9), 33.5 (C-10), 38.7 (C-7), 83.4 (C-5), 95.1 (C-2), 129.2 (C-3), 130.8 (C-4) and 210.3 (C-8); MS, EI [*m/z* (%, composition)]: 196 (2, *M* − 32), 185 (4, $C_9H_{13}O_4$), 151 (8, $C_9H_{11}O_2$), 135 (8, $C_8H_7O_2$), 125 (8, $C_7H_9O_2$), 111 (6, $C_6H_7O_2$), 99 (11, $C_5H_7O_2$), 97 (15, $C_5H_5O_2$ and C_6H_9O), 83 (5, C_5H_7O), 71 (7, C_4H_7O), 55 (9, C_3H_3O) and 43 (100, C_2H_3O and C_3H_7); MS CI (NH_3) [*m/z* (%)]: 246 (3, $M+NH_4$), 228 (4), 195 (64) and 151 (100).

(2*R**,5*R**)-2,5-Epidioxy-2-hydroxy-5-isopropyl-3-nonen-8-one (**6**) was an oil and had $[\alpha]_D$ −0.4° (*c* 0.49, $CHCl_3$); (Found: $[M-32]^{+\cdot}$ 196.1435. Calc. for $C_{12}H_{20}O_2$: 196.1463); IR (CCl_4): 3595, 3484, 1720, 1369, 1356, 1161, 1137, 995, 945 and 911 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.90 (d, *J* 6.8 Hz)/0.95 (d, *J* 7.0 Hz) (H-11/H-12), 1.41 (d, *J* 0.9 Hz, H-1), 1.85 (septet, *J* 7.0 Hz, H-10), 1.90 (ddd, *J* 5.4, 10.7 and −15.0 Hz, H-6a), 2.10 (ddd, *J* 5.0, 10.8 and −15.0 Hz, H-6b), 2.17 (s, H-9), 2.50 (ddd, *J* 5.4, 10.8 and −17.3 Hz, H-7a), 2.65 (ddd, *J* 5.0, 10.7 and −17.3 Hz, H-7b), 3.07 (d, *J* 0.9 Hz, O*H*), 5.84 (d, *J* 10.3 Hz, H-4) and 5.95 (d, *J* 10.3 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 16.9/17.2 (C-11/C-12), 22.8 (C-1), 27.2 (C-6), 30.0 (C-9), 31.2 (C-10), 37.9 (C-7), 82.9 (C-5), 95.7 (C-2), 129.1 (C-3), 129.7 (C-4) and 208.4 (C-8); MS [*m/z* (%, composition)]: 196 (5, *M* − 32), 185 (3, $C_9H_{13}O_4$), 157 (2, $C_8H_{13}O_3$), 151 (7, $C_9H_{11}O_2$), 135 (7, $C_8H_7O_2$), 125 (7, $C_7H_9O_2$), 120 (3, C_9H_{12}), 111 (4, $C_6H_7O_2$), 99 (10, $C_5H_7O_2$), 97 (15, $C_5H_5O_2$ and C_6H_9O), 83 (4, C_5H_7O), 71 (7, C_4H_7O), 55 (8, C_3H_3O and C_4H_7) and 43 (100, C_2H_3O and C_3H_7).

(2*R**,4*S**,5*E*)-2-Hydroxy-7-isopropyl-4-methyl-5-nonen-4-olide (**7**) was an oil and had $[\alpha]_D$ +18° (*c* 0.11, $CHCl_3$); (Found: $M^{+\cdot}$ 226.1502. Calc. for $C_{13}H_{22}O_3$: 226.1569); IR (CCl_4): 3574, 3404, 1790, 1712, 1698, 1378, 1369 and 983 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.78 (t, *J* 7.4 Hz, H-9), 0.80 (d, *J* 6.7 Hz)/0.85 (d, *J* 6.6 Hz) (H-12/H-13), 1.56 (s, H-10), 2.08 (dd, *J* 10.8 and −12.4 Hz, H-3a), 2.68 (dd, *J* 8.1 and −12.4 Hz, H-3b), 4.50 (dd, *J* 8.1 and 10.8 Hz, H-2) and 5.43 (m, H-5 and H-6); ^{1}H NMR (C_6D_6): δ 4.99 (d, *J* 15.6 Hz, H-5) and 5.23 (dd, *J* 8.9 and 15.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 12.3 (C-9), 19.1/20.8 (C-12/C-13), 24.8 (C-8), 28.2 (C-10), 31.5 (C-11), 42.4 (C-3), 50.9 (C-7), 68.6 (C-2), 82.8 (C-4), 132.4/132.5 (C-5/C-6) and 177.0 (C-1); MS [*m/z* (%, composition)]: 226 (0.5, *M*), 211 (0.2, $C_{12}H_{19}O_3$), 208 (0.1), 197 (0.1, $C_{11}H_{17}O_3$), 193 (0.1, $C_{12}H_{17}O_2$), 143 (10, $C_7H_{11}O_3$), 121 (11, C_9H_{13} and C_8H_9O), 109 (66, C_8H_{13}), 97 (35, C_6H_9O and C_7H_{13}), 84 (34, C_6H_{12} and C_5H_8O), 83 (47, C_6H_{11} and C_5H_7O), 69 (29, C_5H_9 and C_4H_5O), 55 (47, C_4H_7 and C_3H_3O) and 43 (100, C_3H_7 and C_2H_3O).

*Acetylation of (2*R*,4*S*,5*E*,7*S*)-2-hydroxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (**2**).* Treatment of 3.3 mg of **2** with 0.2 ml of acetic anhydride in 0.4 ml of pyridine for 4 h at room temperature followed by work-up and purification by HPLC (Spherisorb 5; hexane–EtOAc 40:60) gave 1.1 mg of (2*R*,4*S*,5*E*,7*S*)-2-acetoxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (**9**), which was an oil and had $[\alpha]_D$ +31° (*c* 0.11, $CHCl_3$); IR (CCl_4): 1800, 1753, 1720, 1372 and 1219 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.82 (d, *J* 6.8 Hz)/0.87 (d, *J* 6.7 Hz) (H-14/H-15), 1.57 (s, H-12), 2.11 (dd, *J* 10.6 and −12.6 Hz, H-3a), 2.13 (s, H-11), 2.16 (s, OCOC*H*$_3$), 2.32 (m, H-9a and H-9b), 2.72

(dd, *J* 8.3 and − 12.6 Hz, H-3b) and 5.57 (m, H-2, H-5 and H-6); ^{13}C NMR ($CDCl_3$): δ 19.0/20.6 (C-14/C-15), 20.6 ($OCOCH_3$), 25.7 (C-8), 27.8 (C-12), 30.0 (C-11), 31.8 (C-13), 40.4 (C-3), 41.7 (C-9), 48.5 (C-7), 68.7 (C-2), 82.6 (C-4), 132.3/132.8 (C-5/C-6), 169.7/172.1 (C-1/$OCOCH_3$) and 208.6 (C-10); MS [*m/z* (%)]: 250 (*M* − 60, 2), 232 (3), 217 (2), 207 (1), 189 (3), 174 (9), 147 (9), 135 (4), 119 (8), 109 (6), 95 (9), 93 (9), 81 (6), 69 (8), 55 (10) and 43 (100).

*Preparation of (2*R,*4*S,*5*E,*7*S*)-2-acetoxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (***9***).* A solution of 434 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-6-acetoxy-2,7,11-cembratrien-4-ol (**10**) in 20 ml of dioxane–water (3:1) was stirred with 17 mg of osmium tetraoxide at 0 °C for 10 min. After addition of 1.78 g of sodium periodate the reaction mixture was stirred at room temperature for 5 h, diluted with water, extracted with ethyl acetate, dried and concentrated. Without further purification the crude product, tentatively identified as a mixture of 6-acetoxy-1,4-epoxy-1,5,8-trihydroxy-11-isopropyl-4,8-dimethyl-9-pentadecen-14-ones (**12**) from the ^{1}H NMR spectrum, was dissolved in 10 ml of methanol–water (1:1) and treated with 2.0 g of sodium periodate in 4 ml of water at room temperature for 3 h. Work-up and separation of the crude product by HPLC (Spherisorb 5 Nitrile; hexane–ethyl acetate 60:40) afforded 50.8 mg of an isomeric mixture of hemiacetals **13**. A solution of 3.1 mg of the isomeric mixture (**13**) and 80 mg of PDC in 1 ml of dry dimethylformamide was stirred at room temperature for 26 h. Work-up and purification by HPLC (Spherisorb 5; hexane–ethyl acetate 40:60) gave 1.2 mg of (2*R*,4*S*,5*E*,7*S*)-2-acetoxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (**9**), which was identical (optical rotation, IR, ^{1}H NMR and mass spectra) with the acetate **9** obtained by acetylation of the naturally occurring **2**.

*Preparation of (2*R,*4*R,*5*E,*7*S*)-2-acetoxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (***15***).* A solution of 86 mg of (4*E*,6*R*,8*R*,9*E*,11*S*)-6-acetoxy-8-hydroxy-11-isopropyl-4,8-dimethyl-14-oxo-4,9-pentadecadienal (**16**) in 10 ml dioxane–water (3:1) was stirred with 14 mg of osmium tetraoxide at 0 °C for 10 min. After addition of 296 mg of sodium periodate the reaction mixture was stirred at 0 °C for 3 h and at room temperature for 1 h, diluted with water, extracted with ethyl acetate, dried and concentrated. Without further purification the crude product was dissolved in 5 ml of methanol–water (1:1) and treated with 395 mg of sodium periodate in 1 ml of water at room temperature for 3 h. Work-up and separation of the crude product by HPLC (Spherisorb 5 Nitrile; hexane–ethyl acetate 40:60) afforded 15.4 mg of an isomeric mixture of hemiacetals (**17**). A solution of 8.5 mg of this isomeric mixture (**17**) and 160 mg of PDC in 1 ml of dry dimethylformamide was stirred at room temperature for 24 h. Work-up and purification by HPLC (Spherisorb 5; hexane–ethyl acetate 40:60) gave 5.3 mg of (2*R*,4*R*,5*E*,7*S*)-2-acetoxy-7-isopropyl-4-methyl-10-oxo-5-undecen-4-olide (**15**) which was an oil and had $[\alpha]_D$ − 26° (*c* 0.54, $CHCl_3$); IR (CCl_4): 1796, 1754, 1720, 1374 and 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.83 (d, *J* 6.8 Hz)/0.88 (d, *J* 6.7 Hz) (H-14/H-15), 1.52 (s, H-12), 2.12 (s, H-11), 2.16 (s, $OCOCH_3$), 2.19 (dd, *J* 9.6 and − 12.9 Hz, H-3a), 2.34 (m, H-9a and H-9b), 2.63 (dd, *J* 8.6 and − 12.9 Hz, H-3b), 5.47 (dd, *J* 9.0 and 15.7 Hz, H-6), 5.58 (dd, *J* 8.6 and 9.6 Hz, H-2) and 5.59 (d, *J* 15.7 Hz, H-5); ^{13}C NMR ($CDCl_3$): δ 19.1/20.6 (C-14/C-15), 20.6 ($OCOCH_3$), 25.8 (C-8), 26.8 (C-12), 30.1 (C-11), 31.9 (C-13), 40.5 (C-3), 41.8 (C-9), 48.6 (C-7), 68.7 (C-2), 82.7 (C-4), 132.6 (C-6), 133.9 (C-5), 169.8/171.9 (C-1/$OCOCH_3$) and 208.8 (C-10); MS [*m/z* (%)]: 250 (*M* − 60, 2), 232 (3), 217 (2), 207 (2), 189 (4), 174 (9), 147 (10), 135 (6), 119 (10), 109 (9), 95 (13), 93 (12) 81 (14) 69 (25), 55 (21) and 43 (100).

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