Tobacco Chemistry. 63* Syntheses and Stereostructures of Six Tobacco *Seco*-Cembranoids

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Wahlberg, Inger, Arndt, Rolf, Nishida, Toshiaki and Enzell, Curt R., 1986. Tobacco Chemistry 63.* Syntheses and Stereostructures of Six Tobacco *Seco*-Cembranoids. Acta Chem. Scand. B 40: 123–134.

The isolation of the (4*E*,6*R*,8*S*,9*E*,11*S*)- and (4*E*,6*R*,8*R*,9*E*,11*S*)- 6,8-dihydroxy-4,8-dimethyl-11-isopropyl-14-oxo-4,9-pentadecadienoic acids (*1*, *2*), as their methyl ester (*3*, *4*), from Greek tobacco and of the four (5*E*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olides diastereoisomeric at C-4 and C-8 (*5–8*) from Burley tobacco is reported. Of these, *1* and *2* have previously been found in Burley tobacco, whereas three of the four *seco*-lactones are new natural products. The stereochemistries of *1*, *2* and *5–8* have been determined by chemical correlation with parent cembranoids.

The cembranic diterpenoids, which are present in a substantial amount in the cuticular wax of the leaf and flower of most tobacco varieties, number more than forty compounds to-date.[2] Their presence is of interest from a flavour point of view, since available results suggest that they are prone to undergo biodegradation and capable of giving rise to low-molecular weight odoriferous products. Simple ring cleavage of parent cembranoids to yield acylic compounds could be the first step in these transformations, and a few *seco*-cembranoids are known to occur in tobacco. Among these are two acids, which have been obtained from Burley tobacco and characterized as diastereoisomeric 6,8-dihydroxy-4,8-dimethyl-11-isopropyl-14-oxo-4,9*E*-pentadecadienoic acids (*1*, *2*)[3] and a lactone, also isolated from Burley tobacco, which has been ascribed a 4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (*9*) structure.[4]

Our recent isolation of the two *seco*-acids (*1*, *2*), as their corresponding methyl esters (*3*, *4*), from an extract of flowers of Greek tobacco has encouraged studies on their stereostructures through chemical correlation with parent cembranoids. A similar biomimetic strategy has been used to resolve the absolute stereochemistries of the four *seco*-lactones *5–8* obtained from leaves of air-cured Burley tobacco.

Results

Seco-acids. It can be seen from Table 1 that the ^{13}C NMR spectra of the methyl esters (*3*, *4*) obtained from the two tobacco *seco*-acids (*1*, *2*) differ mainly with respect to the shieldings of C-6, C-8 to C-10 and C-17, suggesting that *1* and *2* (as well as *3* and *4*) are epimeric at C-8. This assignment is also in harmony with the biogenetic argument that *1* and *2* are derived from the two 2,7,11-cembratriene-4,6-diols *10* and *11*, which are the major two tobacco cembranoids.[2]

To confirm this and determine the absolute stereochemistries of the two *seco*-acids (*1*, *2*), the monoacetate *12* of the 4*S*,6*R*-diol *10* was initially treated with osmium tetroxide. The reaction occurs with a stereospecific *cis*-attack on the 11,12 double bond giving a 6-acetoxy-4,11,12-triol (*13*), which exhibits the multiplet due to H-11 at δ 3.28 in its ^1H NMR spectrum.

The stereochemistry of *13* was deduced by chemical means. Thus, the 4,6,11,12-tetrol *14*, which is obtained by alkaline hydrolysis of *13* or

*For part 62 see Ref. 1.

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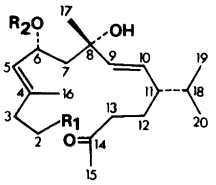

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3 R_1 = COOMe ; R_2 = H *4* R_1 = COOMe ; R_2 = H
16 R_1 = CHO ; R_2 = Ac *20* R_1 = CHO ; R_2 = Ac
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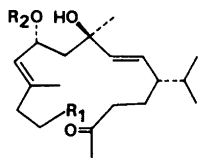

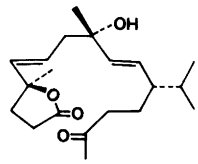

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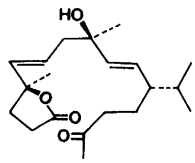

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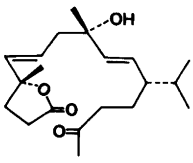

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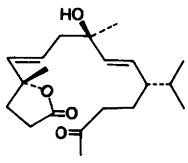

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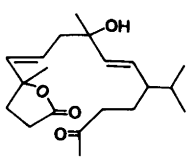

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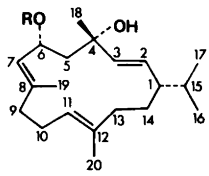

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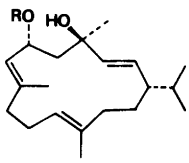

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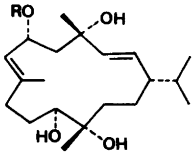

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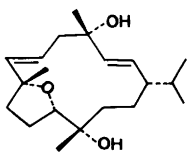

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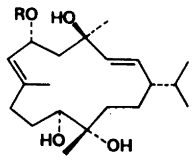

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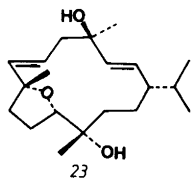

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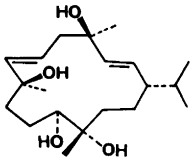

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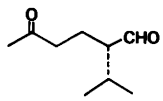

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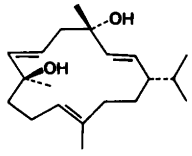

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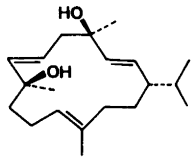

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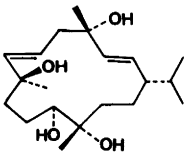

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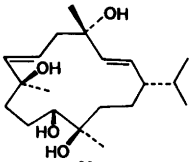

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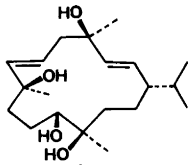

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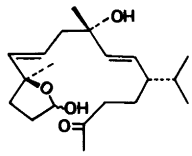

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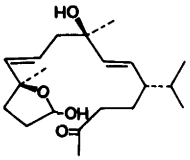

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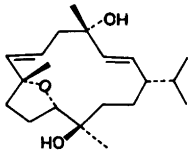

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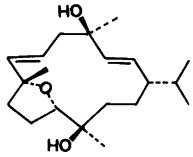

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Table 1. ^{13}C NMR chemical shifts and assignments for compounds *3–8, 13, 14, 16, 19, 22, 24* and *28–30.*[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
3[c]	173.6	34.3	32.5	135.9	128.4[b]	66.2	47.6	73.1	139.9	128.6[b]	48.6	26.2	42.1	209.5	30.0	16.5	26.9	32.0	19.1	20.7
4[d]	173.5	34.2	32.5	136.2	128.1	67.3	47.1	73.8	137.7	129.9	48.8	26.5	42.0	209.4	30.0	16.4	30.9	32.1	19.2	21.0
16[e]	201.7	41.6	31.5	138.5	125.2	68.7	47.2	71.9	138.5	129.6	48.6	26.1	42.0	209.4	30.0	17.0	29.1	31.9	19.0	20.7
20[f]	201.7	41.7	31.4	137.9	124.8	69.7	46.6	72.3	138.1	129.7	48.5	26.0	42.0	209.2	30.0	16.9	29.5	32.0	19.1	20.6
13[g]	49.0	132.1	135.5	72.2	48.4	68.6	125.7	140.9	27.2	36.3	72.2	74.7	34.6	26.4	30.6	20.6[b]	20.8[b]	31.0	15.8	21.0[b]
19[h]	49.4	130.2	136.7	71.5	51.6	68.7	126.4	140.3	23.8	38.0	77.8	74.2	37.5	29.6	31.9	19.9[b]	20.6[b]	29.1	16.5	19.4[b]
14	50.6	130.3	137.1	73.7	47.3	67.1	128.3	138.2	28.2	35.3	74.8	75.0	37.9	26.0	31.8	19.7	20.9	31.7	16.3	20.3
22	50.0	130.7[b]	137.5	72.3	54.2	64.8	130.5[b]	136.9	24.3	38.0	78.2	74.3	37.2	29.8	31.9	19.6[b]	20.8[b]	29.4	16.2	19.4[b]
28	49.8	130.9	137.0	72.8	45.2	124.8	138.3	75.0	37.3	30.0	76.5	74.0	38.0	25.2	32.3	19.7	20.5	30.0	29.4	21.8
29	48.8	131.7	136.9	72.9	45.9	123.2	139.2	75.3	33.1	26.4	77.8	73.1	37.8	26.0	29.3	20.4	21.7	30.6	31.7	22.4
24	49.1	131.7	136.6	72.7	44.3	125.5	138.9	75.1	37.6	27.5	76.4	73.7	39.4	25.2	32.6	19.7	20.5	26.7	30.6	22.7
30	48.8	133.6	136.7	72.9	46.5	123.7	138.9	75.2	33.8	26.2	78.1	73.1	38.8	25.4	29.5	20.3	21.5	25.4	31.5	22.1
5	176.5	28.9	34.2	85.1	136.0	125.2	45.6	72.2	138.3	129.8	48.7	26.2	42.1	209.5	30.1	26.6	28.0	32.0	19.1	20.7
6	176.5	28.9	34.2	85.1	136.0	125.3	45.6	72.3	138.3	129.7	48.7	26.2	42.0	209.3	30.1	26.5	28.4	32.0	19.2	20.7
7	176.5	28.9	34.2	85.1	135.9	125.2	45.7	72.3	138.3	129.7	48.7	26.2	42.1	209.4	30.0	26.6	28.0	32.0	19.1	20.7
8	176.5	28.9	34.2	85.1	136.0	125.3	45.6	72.2	138.3	129.7	48.6	26.2	42.0	209.3	30.1	26.6	28.3	32.0	19.1	20.7

[a] δ-Values in $CDCl_3$ relative to TMS. [b] Assignment may be reversed.
[c] $COOCH_3$: 51.6; [d] $COOCH_3$: 51.6; [e] $OCOCH_3$: 170.3; $OCOCH_3$: 21.5; [f] $OCOCH_3$: 170.0; $OCOCH_3$: 21.4
[g] $OCOCH_3$: 170.3; $OCOCH_3$: 21.4; [h] $OCOCH_3$: 171.6; $OCOCH_3$: 21.5

by the stereoselective reaction of the 4*S*,6*R*-diol *10* with osmium tetroxide, was treated with a trace of hydrochloric acid in chloroform. Several products were formed (*vide infra*), of which one was identified as (1*S*,2*E*,4*S*, 6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*15*).[5] This compound is likely to arise *via* an attack of the hydroxyl group at C-11 on C-8 and elimination of the hydroxyl group at C-6,and its formation is consistent with 11*S*,12*S*-stereochemistries in *13* and *14*.

Oxidation using sodium periodate converted the 6*R*-acetoxy-4*S*,11*S*,12*S*-triol *13* to the *seco*-aldehyde *16*, whose ^{1}H NMR spectrum displayed the prerequisite one-proton triplet and three-proton singlet at δ 9.76 and 2.13, respectively. The *seco*-aldehyde *16* was next reacted with pyridinium dichromate (PDC) and subsequently with ethereal diazomethane to give methyl (4*E*,6*R*,8*S*,9*E*,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (*17*), which was identical to the acetylated methyl ester of the naturally occurring *seco*-acid *1*.

In an analogous reaction sequence, the acetate *18* derived from the 4*R*, 6*R*-diol *11* was converted *via* the acetoxytriol *19* and the *seco*-aldehyde *20* to methyl (4*E*,6*R*,8*R*,9*E*,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (*21*). The latter proved to be indistinguishable from the acetylated derivative of the methyl ester of the tobacco *seco*-acid *2*, hence confirming that the two *seco*-acids are epimeric at C-8.

It is notweorthy that the ^{13}C NMR spectra of the acetoxytriol *19* and the 4*S*,6*R*,11*S*,12*S*-acetoxytriol *13* are vastly different. This would imply that the two compounds differ with respect to the stereochemistry not only at C-4 but also at C-11 and C-12, or that they have different conformations. In order to distinguish between these two possibilities, tetrol *22*, obtained by alkaline hydrolysis of *19* or by osmylation of the 4*R*,6*R*-diol (*11*), was treated with weak acid. Two products were isolated, one being identical to (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*23*).[6] This result demonstrates that *19* has the same 11*S*,12*S*-configuration as *13* and hence that they have different conformations.

The second product (*24*) was identified as a 4,8,11,12-tetrol from its spectral data. It is evidently generated by an allylic rearrangement reaction and must have an 11*S*,12*S*-configuration. In view of previous results for the 4,6-diols (*10,11*)[7] no stereochemistry is assigned to C-4 and C-8 at this stage (*vide infra*).

Seco-lactones. In a search for an authentic sample of the *seco*-lactone *9*,[4] two fractions, *a* and *b*, both having the required spectral data, were isolated from an extract of Burley tobacco. These fractions were not further separable by HPLC but were shown, after silylation and GC on a capillary column, to contain two components each, hence implying that at least four *seco*-lactones (*5–8*) are present in tobacco.

Information on the stereostructures of these was initially obtained by subjecting each fraction (*a,b*) to ozonolysis and subsequent treatment with zinc in acetic acid. The sample of 2-isopropyl-5-oxohexanal (*25*) formed from both fractions was enantiomerically pure and had the expected *S*-configuration.[8] The four seco-lactones (*5–8*) were hence provisionally identified as diasteroisomers with respect to the configuration at C-4 and C-8, a hypothesis that was verified with the aid of biomimetic syntheses.

The first synthetic pathway involved an initial treatment of the 4*S*,8*S*- and 4*R*,8*S*-diols *26* and *27*[7] with osmium tetroxide, which gave two tetrols in each case (*28, 29* and *24, 30*). One of those obtained from the 4*R*,8*S*-diol (*27*) was identical to the aforementioned tetrol *24*, which was therefore attributed a 4*R*, 8*S*,11*S*,12*S*-configuration. As a consequence, *30* was identified as the 4*R*,8*S*,11*R*,12*R*-tetrol, and since a comparison shows that the ^{13}C NMR data can be used for configurational assignments (Table 1), *28* and *29* are ascribed 4*S*,8*S*,11*S*,12*S*- and 4*S*,8*S*,11*R*,12*R*-stereochemistries, respectively. Consistent with this, tetrol *28* proved to be identical to one of the minor products generated on acid-induced rearrangement of the 4*S*,6*R*,11*S*,12*S*-tetrol *14*.

Periodate cleavage of each of the 4*S*,8*S*,11*S*,12*S*- and 4*S*,8*S*,11*R*,12*R*-tetrols (*28, 29*) gave identical mixtures of hemiacetals epimeric at C-1 (*31*). These were oxidized using PDC to give (4*S*,5*E*,8*S*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (*5*). In an analogous manner, the 4*R*,8*S*,11*S*,12*S*- and 4*R*,8*S*,11*R*,12*R*-tetrols *24* and *30* were converted *via* the hemiacetals *32* to the corresponding 8*R*-lactone *6*.

The two complimentary 4*R*,8*S*- and 4*R*,8*R*-lactones *7* and *8* were prepared by a PDC-promoted

oxidative cleavage of the 11,12-bond in the (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*R*)- and (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*R*)-8,11-epoxy-2,6-cembradiene-4,12-diols *33* and *34*.[5]

All four *seco*-lactones (*5–8*) gave rise to virtually identical IR, ^{1}H NMR and mass spectra, and these data agreed well with those of fractions *a* and *b*.

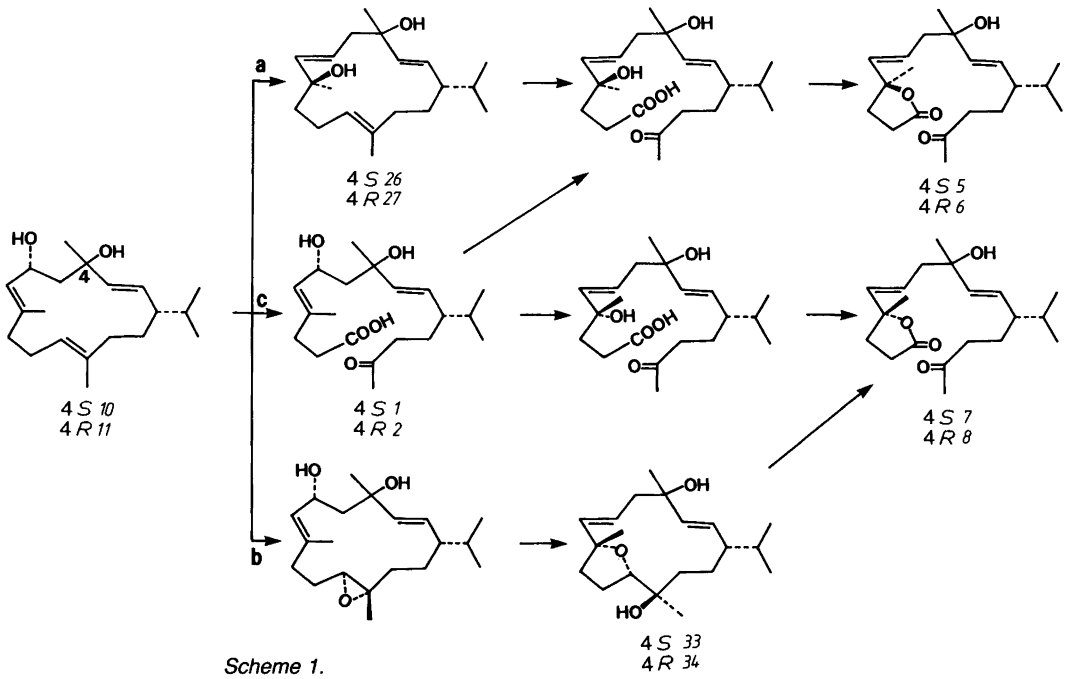
Information on the identities of the components of these two fractions was obtained by co-chromatography with each of the synthetic compounds (*5–8*) on an HPLC column and, after silylation, on a GC capillary column. The results suggested that fraction *a* is a 59:41 mixture of the 4*S*,8*R*- and 4*R*,8*R*-lactones *6* and *8* and that fraction *b* contains the 4*R*,8*S*- and 4*S*,8*S*-lactones *7* and *5* in the ratio 43:57. In conformity with this, the CD spectra of fractions *a* and *b* show virtually no absorption at 212 nm, while the components of each fraction (*6* and *8* / *5* and *7*) give rise to a negative and positive Cotton effect, respectively, at this wavelength due to the $n \rightarrow \pi^*$ transition of the lactone group.

The lactone *9*, previously isolated from tobacco, probably has an 8*S*-configuration. This conclusion rests on a comparison of the chemical shift value reported for its C-17 signal, δ 27.9, with the corresponding values obtained for the lactones *5–8* (*cf.* Table 1). An assignment of the chirality at C-4 in *9*, however, cannot be made in the absence of CD data.

It is reasonable to assume that the lactones *5–8* are formed in tobacco from the two 4,6-diols *10* and *11*. Two of the plausible pathways, a and b in Scheme 1, have been explored synthetically and are described, in part, above. The third route (c), presumably giving both the 4*S*- and 4*R*-lactones (*5–8*), would involve an allylic rearrangement of the preformed *seco*-acids *1* and *2* followed by lactonization.

Experimental

Instruments. Melting points, optical rotations and infrared spectra were recorded on Leitz Wetzlar, Perkin-Elmer 141 and Perkin-Elmer 983 instruments, respectively. ^{1}H and ^{13}C 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. High performance liquid chromatography was carried out using a Waters 6000 A or 510 sol-



Scheme 1.

vent delivery system, a Waters U6K or a Rheodyne 7125 injector and a Waters R-401 differential refractometer. Analytical GC was performed on a Hewlett-Packard 5790 A chromatograph connected to a Hewlett-Packard 3388 A integrator.

Isolation. An extract (250 g), obtained by immersing flowers of *Nicotiana tabacum* (Basma) in chloroform, was partitioned between methanol/water (80:20) and hexane. The methanol phase was concentrated *in vacuo*, dissolved in methanol/ether, dried, filtered and concentrated to give 180 g of a residue. Part of this (16.8 g) was dissolved in ether and extracted with aqueous $NaHCO_3$. The aqueous solution was made acidic, using aqueous HCl, and extracted with ether. The ether solution was dried, treated with ethereal diazomethane overnight and concentrated to give 695 mg of a crude residue.

Flash chromatography over silica gel (ether/dichloromethane/ethyl acetate) separated the crude mixture of methyl esters into nine fractions. Of these, fractions 5 (5 mg), 6 (21 mg) and 8 (39 mg) were subjected to repetitive HPLC using columns packed with Spherisorb 5 and Spherisorb 5 CN and gradients of hexane/ethyl acetate as eluent to give 18.7 mg of methyl (4*E*,6*R*,8*S*,9*E*,11*S*)-6,8-dihydroxy-4,8-dimethyl-11-isopropyl-14-oxo-4,9-pentadecadienoate (*3*) and 9.0 mg of methyl (4*E*,6*R*,8*R*,9*E*,11*S*)-6,8-dihydroxy-4,8-dimethyl-11-isopropyl-14-oxo-4,9-pentadecadienoate (*4*).

A cyclohexane extract of air-cured leaves of Burley tobacco (5.3 g) was separated by flash chromatography over silica gel using a hexane/ethyl acetate gradient as the eluent into four fractions, 1 (4.0 g), 2 (0.42 g), 3 (0.021 g) and 4 (0.44 g). Fraction 2 was separated further by HPLC using columns packed with Spherisorb 5 and Spherisorb 5 CN (hexane/ethyl acetate) to give 5.2 mg of fraction *a* and 13.3 mg of fraction *b*.

Co-chromatography on an HPLC column packed with Spherisorb 5 and on a GC capillary column coated with SE 54 (after silylation) showed that fraction *a* is a 59:41 mixture of the (4*S*,5*E*,8*R*,9*E*,11*S*)- and (4*R*,5*E*,8*R*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olides (*6, 8*). Fraction *b* is a 43:57 mixture of the (4*R*,5*E*,8*S*,9*E*,11*S*)- and (4*S*,5*E*,8*S*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olides (*7, 5*).

Methyl (4*E*,6*R*,8*S*,9*E*,11*S*)-6,8-dihydroxy-4,8-dimethyl-11-isopropyl-14-oxo-4,9-pentadecadienoate (*3*) was an oil and had $[\alpha]_D$ +26° (*c* 0.57, $CHCl_3$) (reported optical rotation: +14°)[3]; (Found: $[M-18]^{+\cdot}$ 350.2482. Calc. for $C_{21}H_{34}O_4$: 350.2457); IR ($CHCl_3$) bands at 3682, 3602, 3482, 1729, 1712, 1604 and 981 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (d, d*J* = 6.7 Hz) / 0.88 (d, *J* = 6.5 Hz) (H-19/H-20), 1.41 (s, H-17), 1.50 (dd, *J* = 2.5 and −14.3 Hz, H-7a), 1.71 (d, *J* = 1.1 Hz, H-16), 1.78 (dd, *J* = 10.0 and −14.3 Hz, H-7b), 2.12 (s, H-15), 2.2–2.5 (overlapping signals due to H-2 and H-13), 2.89 (broad s, -O*H*), 3.67 (s, -COOCH_3), 4.78 (ddd, *J* = 2.5, 8.2 and 10.0 Hz, H-6), 5.24 (dd, *J* = 1.1 and 8.2 Hz, H-5), 5.34 (dd, *J* = 8.6 and 15.3 Hz, H-10) and 5.50 (d, *J* = 15.3 Hz, H-9) (the ^1H NMR data agreed well with those previously published for "methyl ester II a")[3]; MS [*m/z* (%, composition)]: 350 (0.2), 335 (0.2, $C_{20}H_{31}O_4$), 332 (2, $C_{21}H_{32}O_3$), 289 (1, $C_{18}H_{25}O_3$), 231 (1, $C_{15}H_{19}O_2$), 223 (3, $C_{17}H_{19}$), 194 (11, $C_{13}H_{22}O$), 154 (15, $C_9H_{14}O_2$), 136 (25, $C_{10}H_{16}$), 121 (23, C_9H_{13} and C_8H_9O), 109 (12, C_8H_{13} and C_7H_9O), 93 (41, C_7H_9), 79 (21, C_6H_7), 69 (19, C_5H_9 and C_5H_5O), 55 (20, C_4H_7 and C_3H_3O) and 43 (100, C_2H_3O and C_3H_7).

Methyl (4*E*,6*R*,8*R*,9*E*,11*S*)-6,8-dihydroxy-4,8-dimethyl-11-isopropyl-14-oxo-4,9-pentadecadienoate (*4*) was an oil and had $[\alpha]_D$ −32° (*c* 0.37, $CHCl_3$) (reported optical rotation: −36.7°)[3]; (Found: $[M-18]^{+\cdot}$ 350.2335. Calc. for $C_{21}H_{34}O_4$: 350.2457); IR ($CHCl_3$) bands at 3684, 3606, 3486, 1729, 1712, 1604 and 982 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (d, *J* = 6.7 Hz) / 0.92 (d, *J* = 6.8 Hz) (H-19/H-20), 1.27 (s, H-17), 1.49 (dd, *J* = 2.2 and −14.6 Hz, H-7a), 1.63 (d, *J* = 1.0 Hz, H-16), 1.80 (dd, *J* = 10.5 and −14.6 Hz, H-7b), 2.12 (s, H-15), 2.2–2.5 (overlapping signals due to H-2 and H-13), 3.67 (s, −COOCH_3), 4.66 (ddd, *J* = 2.2, 8.5 and 10.5 Hz, H-6),5.22 (dd, *J* = 1.0 and 8.5 Hz, H-5), 5.44 (d, *J* = 15.4 Hz, H-9) and 5.51 (dd, *J* = 8.0 and 15.4 Hz, H-10) (the ^1H NMR data agreed well with those previously reported for "methyl ester II b")[3]; MS [*m/z* (%, composition)]: 350 (0.1), 335 (0.1, $C_{20}H_{31}O_4$), 332 (1, $C_{21}H_{32}O_3$), 289 (1, $C_{18}H_{25}O_3$), 231 (1, $C_{15}H_{19}O_2$), 223 (3, $C_{17}H_{19}$), 194 (8, $C_{13}H_{22}O$), 154 (12, $C_9H_{14}O_2$), 136 (20, $C_{10}H_{16}$), 121 (23, C_9H_{13}), 109 (12, C_8H_{13} and C_7H_9O), 93 (37, C_7H_9), 79 (20, C_6H_7), 69 (19, C_5H_9 and C_4H_5O), 55 (21, C_4H_7 and C_3H_3O) and 43 (100, C_2H_3O and C_3H_7).

Fraction *a* was an oil and had $[\alpha]_D$ −16° (*c* 0.13, EtOH); CD curve (EtOH, 0.75 mg/ml): λ_{max} 280 nm ([θ] = 570), virtually no absorption at 212 nm; IR ($CHCl_3$) bands at 3600, 1768 and 1710 cm^{-1}. The ^1H NMR and mass spectral data agreed well with published data for lactone *9*.[4]

Fraction *b* was an oil and had $[\alpha]_D$ +5.8° (*c* 0.12, EtOH); CD curve (EtOH, 0.75 mg/ml): λ_{max} 280 nm [(θ] = 500), virtually no absorption at 212 nm; IR ($CHCl_3$) bands at 3600, 1768 and 1710 cm^{-1}. The ^1H NMR and mass spectral data agreed well with published data for lactone *9*.[4]

Acetylation of the methyl (4E,6R,8S,9E,11S)- and (4E,6R,8R,9E,11S)-6,8-dihydroxy-4,8-dimethyl-11-isopropyl-14-oxo-4,9-pentadecadienoates (3 and 4). Treatment of 13 mg of *3* with 0.1 ml of acetic anhydride in 0.5 ml of pyridine for 6 h at room temperature followed by work-up and purification by HPLC (Spherisorb 5; hexane/ethyl acetate 1:1) gave 8 mg of methyl (4*E*,6*R*,8*S*,9*E*,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (*17*), which was an oil and had $[\alpha]_D$ −4.2° (*c* 0.81, EtOH); IR ($CHCl_3$) bands at 3681, 3550, 1727 and 1220 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* = 6.7 Hz)/0.88 (d, *J* = 6.7 Hz) (H-19/H-20), 1.27 (s, H-17), 1.75 (d, *J* = 1.3 Hz, H-16), 1.77 (dd, *J* = 6.1 and −14.4 Hz, H-7a), 1.95 (dd, *J* = 6.7 and −14.4 Hz, H-7b), 2.01 (s, $-OCOCH_3$), 2.13 (s, H-15), 3.67 (s, $-COOCH_3$), 5.21 (dd, *J* = 1.3 and 9.2 Hz, H-5), 5.38 (dd, *J* = 8.8 and 15.7 Hz, H-10), 5.49 (d, *J* = 15.7 Hz, H-9) and 5.66 (ddd, *J* = 6.1, 6.7 and 9.2 Hz, H-6); MS [*m/z* (%)]: 350 (M-60, 0.1), 332 (4), 289 (2), 197 (8), 154 (26), 121 (22), 109 (11), 95 (32), 79 (19), 71 (17), 55 (17) and 43 (100).

Acetylation of 6.0 mg of *4* using acetic anhydride/pyridine yielded, after work-up and purification by HPLC (Spherisorb 5; hexane/ethyl acetate 1:1), 2.5 mg of methyl (4*E*,6*R*,8*R*,9*E*, 11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (*21*), which was an oil and had $[\alpha]_D$ −5.7° (*c* 0.21, EtOH); IR ($CHCl_3$) bands at 3679, 3576, 1729 and 1225 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (d, *J* = 6.7 Hz) / 0.89 (d, *J* = 6.7 Hz) (H-19/H-20), 1.26 (s, H-17), 1.68 (dd, *J* = 3.9 and −14.9 Hz, H-7a), 1.73 (d, *J* = 1.3 Hz, H-16), 2.00 (dd, *J* = 8.8 and −14.9 Hz, H-7b), 2.02 (s, $-OCOCH_3$), 2.12 (s, H-15), 3.66 (s, $-COOCH_3$), 5.14 (dd, *J* = 1.3 and 9.0 Hz, H-5), 5.42 (dd, *J* = 8.0 and 15.5 Hz, H-10), 5.45 (d, *J* = 15.5 Hz, H-9) and 5.61 (ddd, *J* = 3.9, 8.8 and 9.0 Hz, H-6); MS [*m/z* (%)]: 350 (M-60, 0.2), 332 (3), 289 (2), 197 (10), 154 (33), 121 (23), 109 (11), 95 (31), 79 (16), 71 (17), 55 (16) and 43 (100).

Preparation of (1S,2E,4S,6R,7E,11S,12S)-6-acetoxy-2,7-cembradiene-4,11,12-triol (13). A solution of 180 mg (0.52 mmol) of *12* in 1 ml of pyridine was added to a stirred and cooled (0 °C) solution of 152 mg (0.60 mmol) of osmium tetroxide in 1 ml of pyridine. After 2 h at room temperature a solution of 300 mg of sodium bisulfite in 3 ml of water was added, and the mixture was stirred for 4 h. Dilution with water and extraction with ethyl acetate afforded 140 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-6-acetoxy-2,7-cembradiene-4,11,12-triol (*13*), which had m.p. 59–60 °C; $[\alpha]_D$ +17° (*c* 0.45, EtOH); IR ($CHCl_3$) bands at 3590, 3448, 1724 and 1250 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, *J* = 6.6 Hz) / 0.89 (d, *J* = 6.6 Hz) (H-16/H-17), 1.05 (s, H-20), 1.31 (s, H-18), 1.68 (d, *J* = 1.0 Hz, H-19), 2.04 (s, $-OCOCH_3$), 3.28 (m, H-11), 5.4–5.9 (overlapping signals due to H-2, H-3, H-6 and H-7); ^1H NMR (C_6D_6): δ 1.94 (dd, *J* = 8.2 and −13.8 Hz, H-5a), 2.13 (dd, *J* = 4.3 and −13.8 Hz, H-5b), 5.53 (d, *J* = 15.4 Hz, H-3), 5.63 (broad d, *J* = 9.1 Hz, H-7), 5.77 (dd, *J* = 8.7 and 15.4 Hz, H-2) and 5.80 (ddd, *J* = 4.3, 8.2 and 9.1 Hz, H-6); MS [*m/z* (%)]: 322 (M-60, 0.2), 304 (5), 286 (1), 261 (3), 243 (2), 227 (3), 206 (3), 139 (12), 121 (26), 109 (23), 95 (29), 81 (34), 71 (37), 55 (26) and 43 (100).

Hydrolysis of (1S,2E,4S,6R,7E,11S,12S)-6-acetoxy-2,7-cembradiene-4,11,12-triol (13). A solution of 8 mg of *13* in 2 ml of methanol and 0.5 ml of aqueous KOH (45 %) was kept at 0 °C and under nitrogen for 1 h. Work-up gave 6 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetrol (*14*) which had m.p. 134–135 °C; $[\alpha]_D$ +6.5° (*c* 1.3, EtOH); IR ($CHCl)_3$ bands at 3682 and 3403 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, *J* = 6.7 Hz) / 0.90 (d, *J* = 6.5 Hz) (H-16/H-17), 1.08 (s, H-20), 1.28 (s, H-18), 1.70 (d, *J* = 1.1 Hz, H-19), 1.84 (dd, *J* = 2.5 and −14.3 Hz, H-5a), 2.09 (dd, *J* = 7.4 and −14.3 Hz, H-5b), 2.32 (broad s, ^-OH), 2.77 (broads s, $-OH$), 3.37 (m, H-11), 3.58 (broad s, $-OH$), 4.62 (ddd, *J* = 2.5, 7.4 and 8.8 Hz, H-6), 5.53 (d, *J*= 15.4 Hz, H-3), 5.64 (dd, *J* = 8.5 and 15.4 Hz, H-2) and 5.70 (dd, *J* = 1.1 and 8.8 Hz, H-7); MS [*m/z* (%)]: 322 (M-18, 0.1), 304 (0.3), 289 (0.2), 279 (0.2), 261 (2), 227 (0.3), 217 (1), 203 (1), 177 (6), 161 (8), 147

(3), 136 (24), 121 (36), 109 (35), 95 (34), 81 (47), 71 (36), 55 (29) and 43 (100).

Treatment of (1S,2E,4S,6R,7E,11E)-2,7,11-cembratriene-4,6-diol (10) with osmium tetroxide. To a cooled (0 °C) solution of 100 mg (0.39 mmol) of osmium tetroxide in 1 ml of pyridine was added a solution of 120 mg (0.39 mmol) of *10* in 1 ml of pyridine. After 2.5 h at room temperature a solution of 200 mg of sodium bisulfite in 5 ml of aqueous pyridine (3:2) was added and the mixture was stirred for 3 h. Work-up and separation by HPLC (Spherisorb 5 ODS; methanol/water 85:15) gave 18 mg of starting material (*10*) and 59 mg of a product, whose IR, ^{1}H NMR and mass spectra were identical to those of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetrol (*14*).

Treatment of (1S,2E,4S,6R,7E,11S,12S)-2,7-cembradiene-4,6,11,12-tetrol (14) with weak acid. A solution of 45 mg of *14* in 2 ml of chloroform was acidified by adding 1.6 ml of chloroform, which was saturated with HCl. The reaction mixture was kept at room temperature for 33 h. Work-up, flash chromatography over silica gel (hexane/ethyl acetate/methanol) and HPLC (Spherisorb 5 CN and Spherisorb 5 ODS) gave 3 mg of a product, $[\alpha]_D$ +17° (*c* 0.16, $CHCl_3$), whose IR, ^{1}H NMR and mass spectra were identical to those of (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*15*), and 0.7 mg of a product, which was indistinguishable from (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*,12*S*)–2,6-cembradiene-4,8,11,12-tetrol (*28*).

Preparation of (4E,6R,8S,9E,11S)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienal (16). A solution of 45 mg (0.21 mmol) of sodium periodate in 0.5 ml of water was added to a solution of 70 mg (0.18 mmol) of *13* in 6 ml of methanol/water (1:1). After 0.5 h at 0 °C, the reaction mixture was diluted with water, extracted with ether, dried and concentrated *in vacuo* to yield 56 mg of (4*E*,6*R*,8*S*,9E,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienal (*16*), which was an oil and had $[\alpha]_D$ −4.8° (*c* 0.27, EtOH); IR ($CHCl_3$) bands at 3460, 2735, 1722 and 1247 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.84 (d, J = 6.7 Hz) / 0.88 (d, J = 6.7 Hz) (H-19/H-20), 1.28 (s, H-17), 1.75 (d, J = 1.3 Hz, H-16), 1.77 (dd, J = 5.9 and −14.4 Hz, H-7a), 1.96 (dd, J = 6.8 and −14.4 Hz, H-7b), 2.01 (s, $-OCOCH_3$), 2.13 (s, H-15), 5.20 (dq, J 1.3 and 9.2 Hz, H-5), 5.36 (dd, J = 8.1 and 15.6 Hz, H-10), 5.49 (d, J = 15.6 Hz, H-9), 5.66 (ddd, J = 5.9, 6.8 and 9.2 Hz, H-6) and 9.76 (t, J = 1.6 Hz, H-1); MS [*m/z* (%)]: 362 (M-18, 0.1), 337 (0.1), 320 (0.4), 302 (6), 277 (0.6), 259 (3), 201 (3), 179 (8), 161 (18), 135 (20), 121 (50), 109 (40), 95 (50), 81 (45), 71 (42), 55 (33) and 43 (100).

Preparation of methyl (4E,6R,8S,9E,11S)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (17). To a solution of 11 mg (0.03 mmol) of *16* in 0.7 ml of dry dimethylformamide was added 35 mg (0.09 mmol) of PDC. After 24 h at room temperature, the reaction mixture was diluted with water, extracted with ether and taken to dryness. The residue was dissolved in ether and treated with ethereal diazomethane at 0 °C for 24 h. Purification by HPLC (Spherisorb 5; hexane/ethyl acetate 20:80) gave 5 mg of methyl (4*E*,6*R*,8*S*,9*E*,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (*17*), which was identical in all respects to the product obtained by methylation and acetylation of the naturally occurring *seco*-acid *1*.

Preparation of (1S,2E,4R,6R,7E,11S,12S)-6-acetoxy-2,7-cembradiene-4,11,12-triol (19). A solution of 510 mg (1.47 mmol) of *18* in 4 ml of pyridine was added to a stirred and cooled (0 °C) solution of 400 mg (1.57 mmol) of osmium tetroxide in 4 ml of pyridine. After 4 h at room temperature a solution of 1.2 g of sodium bisulfite in 6 ml of water was added, and the mixture was stirred for 2 h. Work-up afforded 475 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-6-acetoxy-2,7-cembradiene-4,11,12-triol (*19*), which had m.p. 175–177 °C; $[\alpha]_D$ +31° (*c* 4.0, EtOH); IR ($CHCl_3$) bands at 3600, 3470, 1710 and 1258 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.84 (d, J = 6.5 Hz) / 0.89 (d, J = 6.5 Hz) (H-16/H-17), 1.10 (s, H-20), 1.33 (s, H-18), 1.71 (d, J = 0.8 Hz, H-19), 1.95 (dd, J = 5.8 and −15.1 Hz, H-5a), 2.04 (s, $-OCOCH_3$), 2.06 (dd, J = 2.7 and −15.1 Hz, H-5b), 3.22 (broad s, −O*H*), 3.36 (m, H-11), 5.30 (dd, J = 0.8 and 9.8 Hz, H-7), 5.39 (dd, J = 8.0 and 15.7 Hz, H-2), 5.48 (d, J = 15.7 Hz, H-3) and 5.60 (ddd, J = 2.7, 5.8 and 9.8 Hz, H-6); MS [*m/z* (%)]: 322 (M-60, 0.5), 304 (7), 286 (1), 261 (4), 243 (4), 227 (5), 206 (4), 139 (21), 121 (40), 109 (35), 95 (43), 81 (49), 71 (50), 55 (32) and 43 (100).

Hydrolysis of (1S,2E,4R,6R,7E,11S,12S)-6-acetoxy-2,7-cembradiene-4,11,12-triol (19). A so-

lution of 150 mg (0.39 mmol) of *19* in 1.5 ml of ethanol and 0.2 ml of aqueous potassium hydroxide (45 %) was kept at 0 °C and under nitrogen for 2 h. Work-up and flash chromatography over silica gel (ethyl acetate/methanol 90:10) gave 127 mg of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetrol (*22*) which had m.p. 78–80 °C; $[\alpha]_D$ +16° (*c* 2.0, EtOH); IR ($CHCl_3$) bands at 3599 and 3436 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.84 (d, J = 6.5 Hz) / 0.89 (d, J = 6.5 Hz) (H-16/H-17), 1.09 (s, H-20), 1.39 (s, H-18), 1.72 (d, J = 1.2 Hz, H-19), 1.83 (dd, J = 4.8 and −14.7 Hz, H-5a), 2.04 (dd, J = 3.5 and −14.7 Hz, H-5b), 3.32 (m, $W^{1/2}$ = 10.8 Hz, H-11), 4.73 (ddd, J = 3.5, 4.8 and 9.7 Hz, H-6), 5.26 (dd, J = 8.4 and 16.0 Hz, H-2), 5.33 (dd, J = 1.2 and 9.7 Hz, H-7) and 5.58 (d, J = 16.0 Hz, H-3); MS [*m/z* (%)]: 322 (M-18, 0.1), 304 (0.8), 289 (0.1), 279 (0.2), 261 (2), 243 (0.7), 227 (0.4), 217 (0.5), 206 (0.7), 136 (14), 121 (19), 109 (18), 95 (20), 81 (26), 71 (26), 55 (24) and 43 (100).

*Treatment of (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (11) with osmium tetroxide.* To a cooled (0 °C) solution of 100 mg (0.39 mmol) of osmium tetroxide in 1 ml of pyridine was added a solution of 107 mg (0.35 mmol) of *11* in 1.5 ml of pyridine. After 3 h at room temperature a solution of 200 mg of sodium bisulfite in 3 ml of water was added and the mixture was stirred for 2 h. Work-up and flash chromatography over silica gel (ethyl acetate) gave 78 mg of a product, whose IR, ^1H NMR and mass spectra were indistinguishable from those of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetrol (*22*).

*Treatment of (1*S*,2*E*,4*R*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetrol (22) with weak acid.* A solution of 60 mg of *22*in 2 ml of chloroform was acidified by adding 0.5 ml of chloroform, which was saturated with HCl. The reaction mixture was kept at room temperature for 48 h. Work-up and flash chromatography over silica gel using a hexane/ethyl acetate/methanol gradient yielded 3.7 mg of a product, m.p. 133–134 °C, whose optical rotation, IR, ^1H NMR and mass spectra were identical with those of (1*S*,2*E*,4*R*,6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradiene-4,12-diol (*23*),[6] and 3.4 mg of a product, which was indistinguishable from (1*S*,2*E*,4*R*, 6*E*, 8*S*,11*S*,12*S*)-2,6-cembradiene-4,8.11,12-tetrol (*24*).

*Preparation of (4*E*,6*R*,8*R*,9*E*,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienal (20).* A solution of 192 mg (0.90 mmol) of sodium periodate in 2 ml of water was added to a solution of 300 mg (0.79 mmol) of *19* in 10 ml of methanol/water (1:1). The reaction mixture was kept at 0 °C for 2 h. Work-up gave 256 mg of (4E,6R,8R,9E,*11S*)-*6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienal (20)*, which was an oil and had $[\alpha]_D$ −5.0° (*c* 1.1, EtOH); IR ($CHCl_3$) bands at 3580, 3460, 2729, 1722 and 1250 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (d, J = 6.7 Hz) / 0.89 (d, J = 6.8 Hz) (H-19/H-20), 1.26 (s, H-17), 1.68 (dd, J = 3.8 and −15.0 Hz, H-7a), 1.74 (d, J = 1.2 Hz, H-16), 2.00 (dd, J = 9.0 and −15.0 Hz, H-7b), 2.02 (s, $-OCOCH_3$), 2.12 (s, H-15), 5.13 (dq, J = 1.2 and 8.9 Hz, H-5), 5.3–5.5 (overlapping signals due to H-2 and H-3), 5.61 (ddd, J = 3.8, 8.9 and 9.0 Hz, H-6) and 9.75 (t, J = 1.7 Hz, H-1); MS [*m/z* (%)]: 362 (M-18, 0.1), 337 (0.1), 320 (0.4), 302 (9), 277 (0.8), 259 (4), 201 (4), 179 (10), 161 (19), 135 (22), 121 (51), 109 (42), 95 (57), 82 (48), 71 (40), 55 (36) and 43 (100).

*Preparation of methyl (4*E*,6*R*,8*R*,9*E*,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (21).* To a solution of 128 mg (0.34 mmol) of *20* in 5 ml of dry dimethylformamide was added 1.0 g (2.66 mmol) of PDC. After 18 h at room temperature the reaction mixture was worked up. The residue was dissolved in ether and treated with ethereal diazomethane at 0 °C for 18 h. Purification by HPLC (Spherisorb 5; hexane / ethyl acetate 20:80) furnished, as a minor product, 21 mg of methyl (4*E*,6*R*,8*R*,9*E*,11*S*)-6-acetoxy-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-4,9-pentadecadienoate (*21*), which was identical to the product obtained by methylation and acetylation of the naturally occurring *seco*-acid *2*.

Ozonolysis of fractions a *and* b. A solution of 5 mg of fraction *a* in 0.1 ml of pyridine and 15 ml of methylene chloride was treated with ozone at −65 °C for 35 min. After addition of 500 mg of zinc powder and 2 ml of acetic acid, the reaction mixture was stirred for 2 h, while the temperature was slowly raised to 20 °C. Work-up and separation by HPLC (Spherisorb 5; hexane/ethyl acetate 50:50) gave 0.7 mg of a product whose optical rotation, IR, ^1H NMR and mass spectra were identical to those of 2*S*-isopropyl-5-oxohexanal (*25*).[8]

Fraction *b* (13 mg) was converted to 2.5 mg of

25 on ozonolysis using the conditions described above.

Preparation of the (1S,2E,4S,6E,8S,11S,12S)- and (1S,2E,4S,6E,8S,11R,12R)-tetrols 28 and 29. A solution of 80 mg (0.26 mmol) of *26* in 2 ml of pyridine was added to a stirred solution (0 °C) of 75 mg (0.30 mmol) of osmium tetroxide in 2 ml of pyridine. After 15 min a solution of 150 mg of sodium bisulfite in 1 ml of water was added, and the mixture was stirred for 3.5 h. Work-up and separation by HPLC (Spherisorb 5 ODS; methanol/water 65:35) gave 15 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*S*,12*S*)-2,6-cembradiene-4,8,11,12-tetrol (*28*) and 22 mg of (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*R*)-2,6-cembradiene-4,8,11,12-tetrol (*29*).

(1*S*,2*E*,4*S*,6*E*,8*S*,11*S*,12*S*)-2,6-Cembradiene-4,8,11,12-tetrol (*28*) was an oil and had $[\alpha]_D$ +24° (*c* 0.16, EtOH); IR ($CHCl_3$) bands at 3686, 3600, 3443 and 1602 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (d, J = 6.4 Hz) / 0.88 (d, J = 6.2 Hz) (H-16/H-17), 1.10 (s) / 1.27 (s) / 1.33 (s) (H-18/H-19/H-20), 2.32 (dd, J = 6.5 and −14.2 Hz, H-5a), 2.45 (dd, J = 6.2 and −14.2 Hz, H-5b), 3.71 (m, H-11), 5.41 (d, J = 15.7 Hz, H-3), 5.47 (dd, J = 8.4 and 15.7 Hz, H-2), 5.51 (d, J = 15.9 Hz, H-7) and 5.60 (ddd, J = 6.2, 6.5 and 15.9 Hz, H-6); MS [*m/z* (%)]: 322 (M-18, 0.3), 304 (2), 289 (0.3), 261 (0.7), 243 (0.7), 227 (2), 206 (1), 139 (7), 121 (12), 109 (11), 95 (15), 81 (17), 71 (28), 55 (20) and 43 (100).

(1*S*,2*E*,4*S*,6*E*,8*S*,11*R*,12*R*)-2,6-cembradiene-4,8,11,12-tetrol (*29*) had m.p. 126–128 °C; $[\alpha]_D$ +47° (*c* 1.5, EtOH); IR ($CHCl_3$) bands at 3684, 3602, 3427 and 1604 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, J = 6.5 Hz) / 0.90 (d, J = 6.6 Hz) (H-16/H-17), 1.13 (s) / 1.28 (s) / 1.29 (s) (H-18/H-19/H-20), 3.48 (m, H-11) and 5.3–5.6 (overlapping signals due to H-2, H-3, H-6 and H-7); MS [*m/z* (%)]: 322 (M-18, 0.2), 304 (2), 289 (0.2), 261 (0.7), 243 (0.7), 227 (2), 206 (1), 139 (6), 121 (12), 109 (11), 95 (14), 81 (17), 71 (29), 55 (20) and 43 (100).

Preparation of (4S,5E,8S,9E,11S)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (5). A. A solution of 7 mg (0.033 mmol) of sodium periodate in 1 ml of water was added to a cooled (0 °C) solution of 10 mg (0.029 mmol) of *28* in 2 ml of methanol/water (1:1). The reaction mixture was left for 4 h. Work-up and purification by chromatography over silica gel (hexane/ethyl acetate 20:80) gave a 1:1 mixture of (4*S*,5*E*,8*S*,9*E*,11*S*)-1,8-dihydroxy-4,8-dimethyl-1,4-epoxy-5,9-pentadecadien-14-ones (*31*) epimeric at C-1, which had an IR ($CHCl_3$) band at 1710 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.26/1.44 (H-16), 1.27 (H-17) and 2.12/2.13 (H-15); MS [*m/z* (%)]: 320 (M-18, 0.1), 302 (0.7), 284 (0.1), 259 (0.2), 241 (0.1), 215 (0.3), 201 (0.6), 197 (13), 179 (4), 161 (10), 139 (6), 121 (28), 109 (17), 95 (20), 82 (23), 71 (22), 55 (15) and 43 (100).

B. Treatment of the 4*S*,8*S*,11*R*,12*R*-tetrol *29* with sodium periodate in methanol/water afforded the same isomeric mixture of hemiacetals (*31*) as that obtained from the 4*S*,8*S*,11*S*,12*S*-tetrol *28*.

A solution of 9.4 mg (0.028 mmol) of the isomeric mixture *31* and 15 mg (0.040 mmol) of PDC in 1 ml of freshly distilled and dried dimethylformamide was stirred at room temperature for 3 h. Work-up and chromatography over silica gel (hexane/ethyl acetate 20:80) gave 5.2 mg of (4*S*,5*E*,8*S*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (*5*), which was an oil and had $[\alpha]_D$ +8.6° (*c* 0.37, EtOH); CD curve (EtOH, 1.0 mg/ml): λ_{max} 278 nm ($[\theta] = 6.1 \cdot 10^2$); λ_{max} 213 nm ($[\theta] = 1.5 \cdot 10^3$); IR ($CHCl_3$) bands at 3598, 1768 and 1709 cm^{-1}. The ^1H and ^{13}C NMR and mass spectral data were good agreement with published data for the tobacco *seco*-lactone *9*.[4]

Preparation of the (1S,2E,4R,6E,8S,11S,12S)- and (1S,2E,4R,6E,8S,11R,12R)-2,6-cembradiene-4,8,11,12-tetrols 24 and 30. A solution of 40 mg (0.13 mmol) of *27* in 2 ml of pyridine was added to a stirred and cooled (0 °C) solution of 40 mg (0.16 mmol) of osmium tetroxide in 2 ml of pyridine. After 15 min a solution of 150 mg of sodium bisulfite in 1 ml of water was added and the mixture was stirred for 4 h. Work-up and separation by HPLC (Spherisorb 5 ODS; methanol/water 65:35) followed by flash chromatography over silica gel (ethyl acetate/methanol 90:10) gave 8 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*R*)-2,6-cembradiene-4,8,11,12-tetrol (*30*) and 6 mg of (1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-2,6-cembradiene-4,8,11,12-tetrol (*24*).

(1*S*,2*E*,4*R*,6*E*,8*S*,11*S*,12*S*)-2,6-Cembradiene-4,8, 11,12-tetrol (*24*) had m.p. 170–172 °C; $[\alpha]_D$ +29° (*c* 0.28, EtOH); IR ($CHCl_3$) bands at 3684, 3602, 3440 and 1602 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (d, J = 6.6 Hz) / 0.87 (d, J = 6.5 Hz) (H-16/H-17), 1.10 (s) / 1.28 (s) / 1.35 (s) (H-18/H-19/H-20), 2.39 (dd, J = 5.0 and −14.5 Hz, H-5a), 2.50

(dd, J = 7.2 and −14.5 Hz, H-5b), 3.64 (m, H-11), 5.31 (dd, J = 8.2 and 16.1 Hz, H-2), 5.44 (d, J = 16.1 Hz, H-3), 5.52 (d, J = 16.0 Hz, H-7) and 5.58 (ddd, J = 5.0, 7.2 and 16.0 Hz, H-6); MS [*m/z* (%)]: 322 (M-18, 0.1), 304 (1), 286 (0.6), 261 (0.9), 243 (1), 227 (2), 206 (2), 139 (11), 121 (18), 109 (18), 95 (20), 81 (24), 71 (31), 55 (19) and 43 (100).

(1*S*,2*E*,4*R*,6*E*,8*S*,11*R*,12*R*)-2,6-Cembradiene-4,8,11,12-tetrol (*30*) had m.p. 88–91 °C; $[\alpha]_D$ +32° (*c* 0.28, EtOH); IR ($CHCl_3$) bands at 3682, 3601, 3421 and 1602 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.83 (d, J = 6.6 Hz) / 0.90 (d, J = 6.6 Hz) (H-16/H-17), 1.12 (s) / 1.30 (s) / 1.39 (s) (H-18/H-19/H-20), 3.43 (m, H-11) and 5.3–5,6 Hz (overlapping signals due to H-2, H-3, H-6 and H-7); MS [*m/z* (%)]: 322 (M-18, 0.2), 304 (2), 286 (0.4), 261 (1), 243 (1), 227 (2), 206 (1), 139 (12), 121 (20), 109 (19), 95 (20), 81 (25), 71 (32), 55 (20) and 43 (100).

*Preparation of (4*S*,5*E*,8*R*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (6). A.* A solution of 5.0 mg (0.023 mmol) of socium periodate in 1 ml of water was added to a cooled (0 °C) solution of 6.0 mg (0.018 mmol) of *30* in 2 ml of methanol/water (1:1). The reaction mixture was left for 2 h. Work-up and chromatography over silica gel (ethyl acetate) gave 3.0 mg of a 1:1 mixture of (4*S*,5*E*,8*R*,9*E*,11*S*)-1,8-dihydroxy-4,8-dimethyl-1,4-epoxy-5,9-pentadecadien-14-ones (*32*) epimeric at C-1, which had an IR ($CHCl_3$) band at 1711 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.26/1.45 (H-16), 1.27/1.28 (H-17) and 2.12/2.13 (H-15); MS [*m/z* (%)]: 302 (M-36, 1), 284 (0.1), 259 (0.2), 241 (0.2), 215 (0.4), 201 (0.8), 197 (10), 179 (3), 161 (9), 139 (4), 121 (20), 109 (15), 95 (21), 82 (17), 71 (18), 55 (19) and 43 (100).

B. Treatment of the 4*R*,8*S*,11*S*,12*S*-tetrol *24* with sodium periodate in methanol/water gave the same epimeric mixture of hemiacetals (*32*) as that obtained from the 4*R*,8*S*,11*R*,12*R*-tetrol *30*.

A solution of 3.0 mg (0.009 mmol) of the isomeric mixture *32* and 6.0 mg (0.016 mmol) of PDC in 1.5 ml of freshly distilled and dried dimethylformamide was stirred at room temperature for 9 h. Work-up and chromatography over silica gel (hexane/ethyl acetate 40:60) yielded 2.7 mg of (4*S*,5*E*,8*R*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (*6*), which was an oil and had $[\alpha]_D$ −13° (*c* 0.21, EtOH); CD curve (EtOH, 1.0 mg/ml): λ_{max} 279 nm ($[\theta] = 6.1 \cdot 10^2$); λ_{max} 210 nm ($[\theta] = -2.5 \cdot 10^3$); IR ($CHCl_3$) bands at 3602, 1768 and 1711 cm^{-1}. The ^1H NMR and mass spectra agreed well with published data for the tobacco *seco*-lactone 9.[4]

Preparation of (4R,5E,8S,9E,11S)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (7). A solution of 12.8 mg (0.040 mmol) of *33* and 90 mg (0.24 mmol) of PDC in 2 ml of dimethylformamide was stirred at room temperature for 24 h. Work-up and chromatography over silica gel (hexane/ethyl acetate 20:80) gave 4.4 mg of starting material (*33*) and 4.7 mg of (4*R*,5*E*,8*S*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (*7*), which was an oil and had $[\alpha]_D$ +2.3° (*c* 0.51, EtOH); CD curve (EtOH, 1.0 mg/ml): λ_{max} 280 nm ($[\theta] = 5.0 \cdot 10^2$); λ_{max} 210 nm ($[\theta] = 1.5 \cdot 10^3$); IR ($CHCl_3$) bands at 3602, 1766 and 1710 cm^{-1}. The ^1H and ^{13}C NMR and mass spectral data agreed well with published data for the tobacco *seco*-lactine 9.[4]

Preparation of (4R,5E,8R,9E,11S)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide ('). A solution of 17 mg (0.053 mmol) of *34* and 120 mg (0.32 mmol) of PDC in 2 ml of dimethylformamide was stirred at room temperature for 16 h. Work-up and purification by HPLC (Spherisorb 5 CN; hexane/ethyl acetate 50:50) gave 2.2 mg of (4*R*,5*E*,8*R*,9*E*,11*S*)-4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-5,9-pentadecadien-4-olide (*8*), which was an oil and had $[\alpha]_D$ −16° (*c* 0.25, EtOH); CD curve (EtOH, 1.0 mg/ml): λ_{max} 280 nm ($[\theta] = 6.6 \cdot 10^2$); λ_{max} 212 nm ($[\theta] = 2.2 \cdot 10^3$); IR ($CHCl_3$) bands at 3600, 1766 and 1711 cm^{-1}. The ^1H NMR and mass spectral data agreed well with published data for the tobacco *seco*-lactone 9.[4]

Acknowledgements. We are grateful to Dr Petra Ossowski-Larsson and Mr Jacek Bielawski for recording the mass spectra.

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Received April 26, 1985.