Tobacco Chemistry. 69.* Five New Labdanic Compounds from Tobacco

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The isolation of (12*S*,13*E*)-13-labdene-8,12,15-triol (**1**), (12*R*,13*E*)-8,12-epoxy-13-labden-15-ol (**2**) and the corresponding 12*S*-epimer (**3**), (11*S*,12*R*)-11,12-epoxy-8-hydroxy-14,15-dinor-13-labdanone (**4**) and 14,15-dinor-8-labdene-7,13-dione (**5**) from Greek tobacco is reported. Of these, **1**, **2**, **4** and **5** are new natural products, **2** and **5** having previously been described as synthetic products. Compound **3** is new to tobacco. The structures of **1** and **4** have been determined using spectroscopic and chemical methods and, in the case of **4**, also via X-ray analysis of (11*R*,12*R*,13*S*)-11,12-epoxy-14,15-dinor-8,13-labdanediol (**21**). The biogenesis of **1–5** is discussed.

Previous studies have shown that the cuticular wax of the leaf and flower of certain tobacco varieties such as Greek and Turkish tobaccos contains substantial amounts of diterpenoids of the labdane class. These compounds are prone to biodegradation by processes ultimately involving loss of carbon atoms, thereby forming odoriferous products containing 14 to 19 carbon atoms.[2] We now report the isolation of three new labdanic diterpenoids (**1–3**) and two new labdane-derived C_{18} compounds (**4**, **5**), four (**1–4**) from flowers and one (**5**) from sun-cured leaves of Greek tobacco.

Results

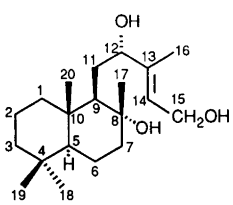
It was concluded from the spectral data that the first new compound (**1**), $C_{20}H_{36}O_3$, contains five methyl groups and one trisubstituted double bond. The oxygen atoms are present as three hydroxy groups, of which one is primary, one is secondary and one is tertiary. These results are consonant with a carbobicyclic structure, and it seemed most likely from a biogenetic point of view that triol **1** is a diterpenoid of the labdane class.

This assignment was supported by a comparison which revealed that fourteen signals in the ^{13}C NMR spectrum of triol **1** were of appropriate multiplicities and had chemical shift values close to those of the C-1 to C-10 and C-17 to C-20 signals for (12*S*)-13(16),14-labdadiene-8,12-diol (**6**).[3] The tertiary hydroxy group in triol **1** was hence assigned to C-8, while the remaining two hydroxy groups and the double bond must be present in the side-chain.

$^1H-^1H$ and $^1H-^{13}C$ shift correlation spectroscopy was used to incorporate the primary hydroxy group and the double bond in partial structure **A**. Since the proton under the secondary hydroxy group resonated as a doublet of doublets in the ^1H NMR spectrum, **A** was extended to the partial structure **B**. Triol **1** was consequently identified as a 13-labdene-8,12,15-triol.

The 13,14 double bond in triol **1** was attributed an *E*-geometry from NOE measurements involving the methyl group on C-13 (H-16) and the protons of the primary hydroxy group (H-15). A

*For part 68 see Ref. 1.



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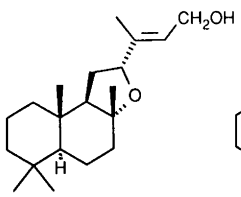

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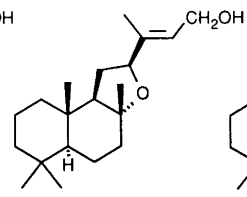

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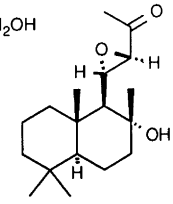

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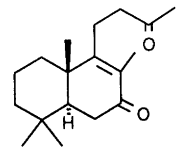

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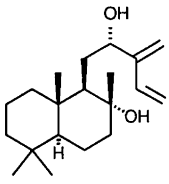

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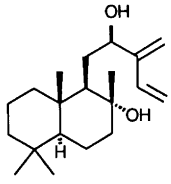

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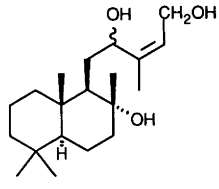

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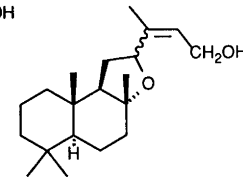

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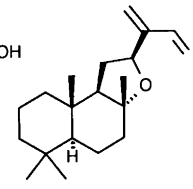

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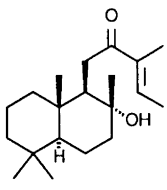

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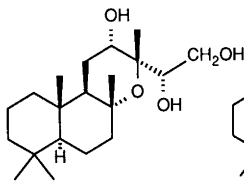

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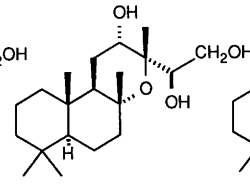

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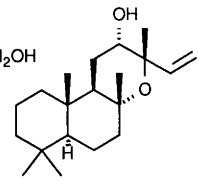

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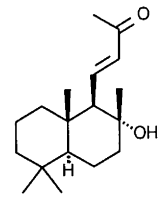

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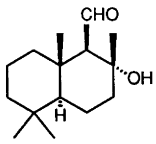

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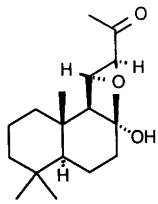

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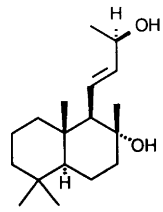

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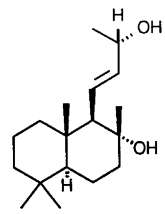

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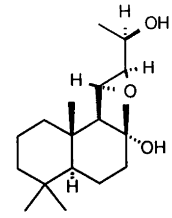

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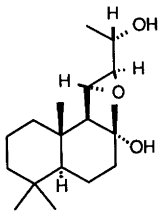

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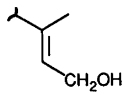

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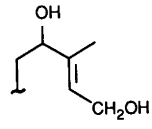

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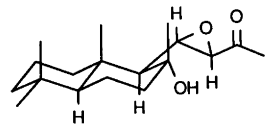

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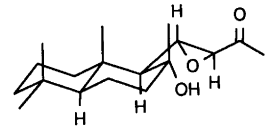


D

clue to the configuration of C-12 was provided by a comparison of the ^{13}C NMR spectra of triol **1**[4] and the 8,12-diols **6** and **7**. It can be seen in Table 1 that C-9 resonates at δ 58.7 and 59.0 in triol **1** and the 8,12*S*-diol **6**, respectively, but at δ 54.4 in the 8,12*R*-diol **7**.

As a means of confirming the proposed 12*S*-configuration, an attempt was made to convert triol **1** into (12*S*,13*E*)-8,12-epoxy-13-labden-15-ol (**3**) by treatment with stannic chloride in chloroform, precedents being the S_N1 type of conversions of (13*Z*)-13-labdene-8,12,15-triol (**8**) into an epimeric mixture of (13*Z*)-8,12-epoxy-13-labden-15-ols (**9**),[5] and of **6** to (12*S*)-8,12-epoxy-13(16),14-labdadiene (**10**).[3] Under these conditions, however, triol **1** did not give **3** but (13*E*)-8-hydroxy-13-labden-12-one (**11**) [IR: 1665 cm^{-1}. ^1H NMR: δ 6.82 (H-14), 1.87 (H-15) and 1.79 (H-16). ^{13}C NMR: δ 14.8 (C-15) and 11.6 (C-16)[6]]. This result may be rationalized as outlined in Scheme 1 by loss of the hydroxy group at C-15 and of H-12 followed by tautomerization, a probable driving force being the generation of a thermodynamically stable α,β-unsaturated ketone.

Using a trace of H_2SO_4 in dioxane–water, the reaction followed a different course, and (12*R*,13*E*)-8,12-epoxy-13-labden-15-ol (**2**) was isolated as the sole product. If triol **1** has a 12*S*-configuration, this outcome may be accounted for by triol **1** undergoing an S_N2-type process involving a proton-induced loss of the hydroxy group at C-12 and attack of the 8-hydroxy group on C-12 (Scheme 1).

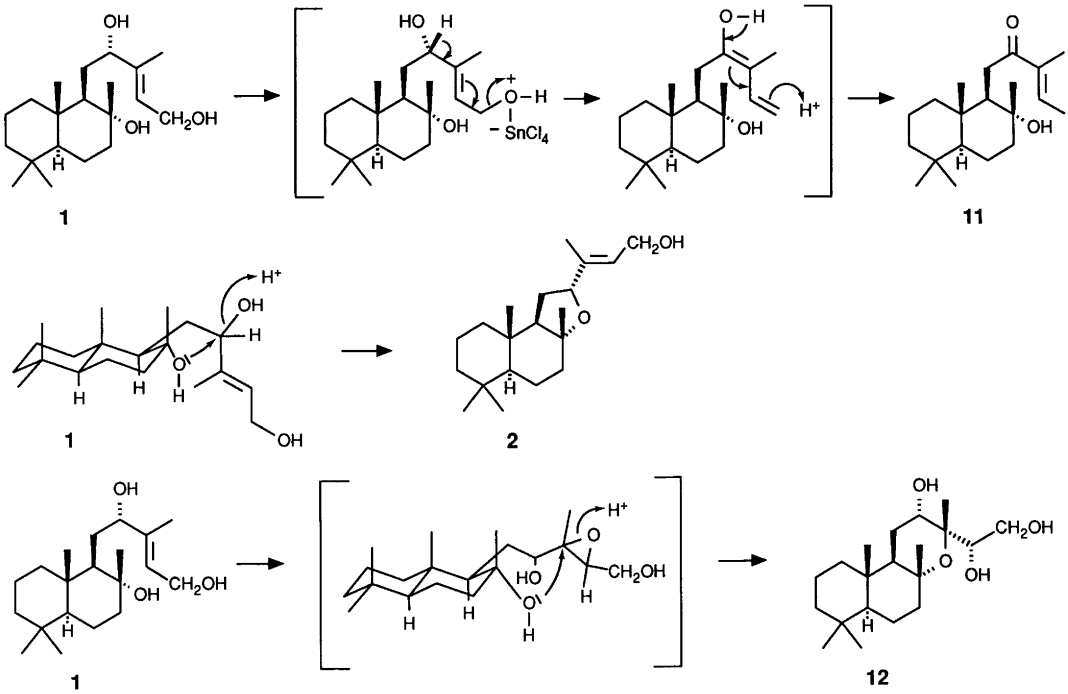
Conclusive stereochemical evidence was obtained by treatment of triol **1** with *m*-chloroperbenzoic acid in chloroform. The major product formed proved to be identical with the major (**12**) of the two 14-isomers of (12*S*,13*R*)-8,13-epoxy-labdane-12,14,15-triols (**12**, **13**) prepared by osmylation of (12*S*,13*S*)-8,13-epoxy-14-labden-12-ol (**14**). As outlined in Scheme 1, this finding is only consistent with triol **1** having a 12*S*-configuration. The intermediate epoxide, if formed, has a 13*S*,14*S*-stereochemistry and undergoes an S_N2-type reaction in which the 8-hydroxy group attacks C-12. The resultant 12,14,15-triol (**12**) must then have a 12*S*,13*R*,14*S*-stereochemistry.

The second and third new tobacco constituents were identified as the (12*R*,13*E*)- and (12*S*,13*E*)-8,12-epoxy-13-labden-15-ols (**2**, **3**) by

Table 1. ^{13}C NMR chemical-shift values and assignments for compounds **1**, **4–7**, **11–13**, **15** and **17–21**.[a]

	Carbon																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	39.3	18.4	41.9	33.3	56.0	20.5	44.0	73.2	58.7	39.3	32.0	78.4	141.8	123.3	59.2	12.6	25.0	33.4	21.4	15.3
4	40.2	18.2	41.7	33.2	55.3	20.5	44.9	72.5	62.5	38.7	56.1	58.6	206.3			25.8	25.6	33.4	21.7	16.0
5	35.9	18.5	41.3	33.1	50.4	35.2	200.0	130.4	166.8	41.1	22.7	42.4	206.9			29.8	11.4	32.5	21.3	17.9
6	39.5	18.4	41.9	33.3	56.0	20.5	44.1	73.2	59.0	39.3	33.6	72.9	150.3	136.6	113.9	113.5	25.0	33.4	21.4	15.4
7	39.5	18.5	41.9	33.5	56.0	20.5	45.0	73.9	54.4	38.5	31.2	69.8	149.5	137.1	113.7	114.8	24.6	33.5	21.6	15.7
11	39.3	18.4	41.8	33.2	55.9	20.6	44.7	73.1	56.0	38.6	32.7	203.0	138.3	136.5	14.8	11.6	23.2	33.4	21.4	15.8
12	38.7	18.5	42.0	33.3	56.4	19.8	42.6	76.1	49.6	36.3	23.1	70.7	76.9	76.3	62.5	22.2	24.7	33.3	21.2	15.8
13	38.6	18.4	42.0	33.3	56.4	19.8	42.7	76.1	49.0	36.3	23.3	73.1	78.2	76.0	63.4	24.8[b]	24.9[b]	33.3	21.2	16.0
15	41.0	18.4	41.8	33.4	55.6	20.2	43.1	72.4	65.9	37.9	144.8	135.5	197.7			27.6	24.9	33.4	21.6	16.0
17	40.5	18.3	41.6	33.1	55.5	19.9	42.2	73.5	61.2	37.8	57.5	60.9	205.0			25.0	25.6	33.5	21.7	16.0
18	40.8	18.4	41.9	33.3	55.8	20.1	42.2	71.7	65.6	37.3	140.4	125.8	68.5			23.7	25.0	33.4	21.6	15.8
19	40.7	18.4	41.9	33.3	55.7	20.1	42.6	72.1	65.6	37.3	140.3	126.4	69.0			23.7	24.7	33.4	21.6	15.9
20	40.5	18.3	41.6	33.1	55.5	19.8	42.2	73.8	60.5	37.8	55.2	63.0	64.9			18.8	25.7	33.5	21.7	16.0
21	40.5	18.3	41.7	33.1	55.5	19.8	42.1	73.7	60.6	37.7	56.8	64.1	66.9			20.0	25.6	33.5	21.7	16.0

[a]δ-Values in CDCl$_3$ relative to SiMe$_4$. [b]Assignment may be reversed.



Scheme 1. Proposed mechanisms for the conversion of **1** into **11**, **2** and **12**.

comparison with corresponding authentic samples, which have previously been prepared synthetically.[7] The 12*R*-isomer (**2**) is apparently a new natural product, while the 12*S*-isomer (**3**), carterochaetol, has previously been isolated from *Carterothamnus anamalochaeta*.[8]

Compound **4**, $C_{18}H_{30}O_3$, contains a methyl ketone group [IR band at 1709 cm^{-1}; ^1H NMR methyl singlet at δ 2.17] and a tertiary hydroxy group [OH absorption in the IR spectrum; ^{13}C NMR signal at δ 72.5 (s)]. The remaining oxygen atom in **4** is present as a 1,2-epoxide group [^1H NMR signal at δ 3.02 (dd) and 3.67 (d)]. These results and the absence of signals due to olefinic carbon atoms in the ^{13}C NMR spectrum were consonant with **4** being carbobicyclic. Useful structural information was also provided by a comparison of the ^{13}C NMR spectra of **4** and (*E*)-14,15-dinor-8-hydroxy-11-labden-13-one (**15**)[9] (Table 1). Thus, since twelve of the signals for **4** were of appropriate multiplicities and had chemical shift values close to those found for the C-1 to C-6, C-8, C-10, and C-17 to C-20 signals for **15**, it seemed likely that **4** is an 11,12-epoxide of **15**.

This assignment was readily verified by treatment of **15** with hydrogen peroxide in alkali, which afforded the two expected 11,12-epoxides in the ratio 3:2. A small amount of 8-hydroxy-11-drimanal (**16**)[9] formed via a retro-aldol reaction was also obtained. The minor 11,12-epoxide was identical in all respects with the naturally occurring **4**.

The two epoxides (**4**, **17**) were subjected to transient NOE experiments. These involved the irradiation of H-11, and led, for both compounds, to enhancements of the signals due to the methyl groups at C-8 and C-10 (H-17 and H-20), while irradiation of H-12 enhanced the signal due to H-9. These results suggest that the side-chain in both compounds have conformations of types **C** and **D**. The coupling constants observed between H-9 and H-11, J = 8.8 Hz for **4** and 9.4 Hz for **17**, are consistent with such orientations.

In contrast with the case of **4**, the hydroxy hydrogen in epoxide **17**, giving rise to a signal at δ 3.34 in the ^1H NMR spectrum which is not shifted on dilution, participates in hydrogen-bonding with the epoxide oxygen. This result

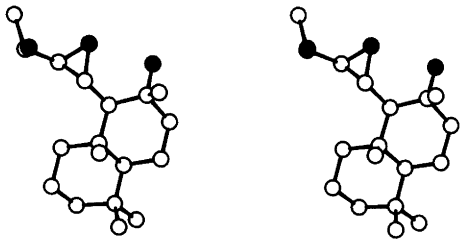


Fig. 1. A stereoscopic view of (11*R*,12*R*,13*S*)-11,12-epoxy-14,15-dinor-8,13-labdanediol (**21**).

may be rationalized if **17** has an 11*R*,12*S*- and **4** an 11*S*,12*R*-stereochemistry. This assignment was confirmed by the conversion of **15** into the two 8,13-diols **18** and **19** by using $NaBH_4$. Treatment of diol **19** with t-butyl hydroperoxide and a catalytic amount of vanadyl acetylacetonate or with *m*-chloroperbenzoic acid proceeded with a stereoselective attack on the 11,12 double bond. The 11,12-epoxide thus formed (**21**) was subjected to X-ray analysis.

Compound **21** crystallized in the monoclinic space group $P2_1$. The crystal data, obtained on a Philips PW 1100 diffractometer, were $a = 22.169$, $b = 10.588$, $c = 8.020$ Å; $\beta = 110.73°$; $Z = 4$. The normal *R*-value based on refinement including anisotropic thermal parameters for all non-hydrogen atoms is 0.128. Work to locate the hydrogen atoms and further refinement is underway.[10] A stereoscopic view, which summarizes the X-ray results and demonstrates that **21** is (11*R*,12*R*,13*S*)-11,12-epoxy-14,15-dinor-8,13-labdanediol, is shown in Fig. 1.

Oxidation of **21** gave (11*R*,12*S*)-11,12-epoxy-8-hydroxy-14,15-dinor-13-labdanone, which proved to be identical with **17**. The naturally occurring **4** is hence conclusively identified as the corresponding (11*S*,12*R*)-epimer. The epoxidation of the 11,12 double bond in the 8,13*R*-diol **18** is also stereoselective giving (11*R*,12*R*,13*R*)-11,12-epoxy-14,15-dinorlabdane-8,13-diol **20**, which is converted into **17** on being oxidized. This stereoselectivity may be accounted for by conformational factors and a directing effect exerted by the hydroxy group at C-8.

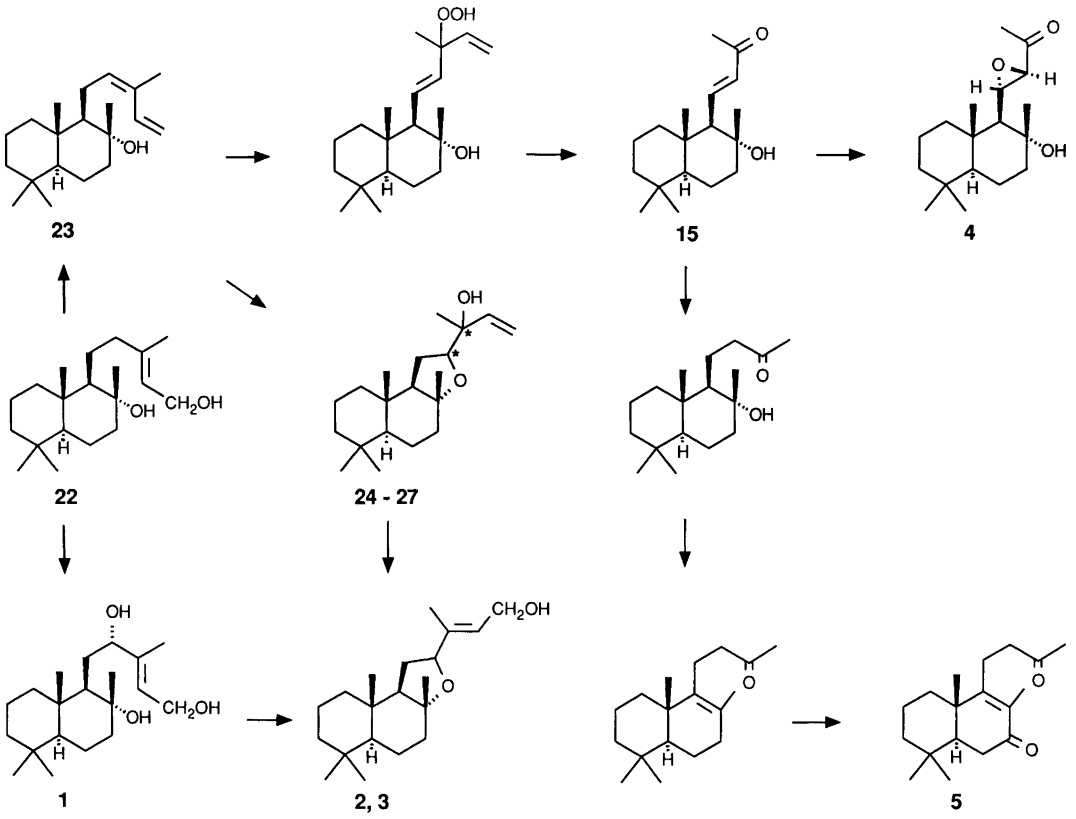
The fifth isolate, 14,15-dinor-8-labdene-7,13-dione (**5**), previously reported as a product of the oxidation of sclareol with chromic acid,[11] is a new tobacco constituent and, to our knowledge, also a new natural product.

The biogenesis of the new tobacco constituents **1–5** is suggested to have the course outlined in Scheme 2. (*E*)-13-Labdene-8,15-diol (**22**), derived from geranylgeranyl pyrophosphate, undergoes allylic oxidation to form triol **1**. Compounds **2** and **3** may arise via cyclization of triol **1** or via oxidation of (*Z*)-abienol (**23**) to the 8,12-epoxy-14-labden-13-ols (**24–27**) and subsequent allylic rearrangement. (*Z*)-Abienol (**23**), which is the major labdanoid in the green leaf and fresh flower of tobacco,[12] is also a plausible precursor of the new C_{18} constituents **4** and **5**. Oxidation and a subsequent Hook degradation accounts for the formation of the enone **15**,[3] which in turn gives rise to **4** by epoxidation and to **5** by hydrogenation, dehydration and allylic oxidation. Support for the validity of these pathways is provided by the fact that compounds **15** and **22–27** are tobacco constituents.[9,12–14]

Experimental

Optical rotations were recorded on a Perkin–Elmer 241 polarimeter and part of the high performance liquid chromatography work was carried out using a Waters Delta Prep 3000 solvent delivery system, a Waters U6K injector and a Waters R-403 differential refractometer. For other instrumental details see Ref. 15.

Isolation. An extract (200 g) obtained by immersing flowers of Greek tobacco (Basma) in chloroform was initially separated by flash chromatography over silica gel using hexane–EtOAc gradient elution into seven fractions, 1 (15 g), 2 (7.0 g), 3 (3.8 g), 4 (9.5 g), 5 (21 g), 6 (85 g), and 7 (11 g). Fraction 3 was separated further by flash chromatography (SiO_2; hexane–EtOAc gradient) into seven fractions, 31–37. Fraction 35 (710 mg) was subjected to repeated HPLC using columns packed with Lichrosorb 10 Diol, Spherisorb 5 CN and Spherisorb 5 (hexane–EtOAc 70:30) to give 2.2 mg of (11*S*,12*R*)-11,12-epoxy-8-hydroxy-14,15-dinor-13-labdanone (**4**). Part (8 g) of fraction 5 was separated by flash chromatography (SiO_2; hexane–EtOAc gradient) into fractions 51–55. Fraction 52 (485 mg) was fractionated further by HPLC (Lichrosorb 10 Diol and Spherisorb 5 CN; hexane–EtOAc 60:40) to give 1.3 mg of (12*R*,13*E*)-8,12-epoxy-13-labden-15-ol (**2**) and 2.1 mg of (12*S*,13*E*)-8,12-epoxy-13-labden-15-ol (**3**).



Scheme 2. Proposed biogenesis of **1–5**.

Fraction 6 (128 g) was separated by flash chromatography (SiO_2; hexane–EtOAc gradient) into eight fractions, 61–68. Fraction 65 (26.8 g) was separated further by HPLC (Spherisorb 5 CN; hexane–EtOAc 40:60) into three fractions 65A (17.1 g), 65B (8.0 g), and 65C (0.57 g). Part of fraction 65C (110 mg) was purified by HPLC (Spherisorb 5 CN; EtOAc) to give 46 mg of (12*S*,13*E*)-13-labdene-8,12,15-triol (**1**).

14,15-Dinor-8-labdene-7,13-dione (**5**, 2.3 mg) was isolated from fraction B7 of a diethyl ether extract obtained from 295 kg of Greek tobacco[16] by column chromatography over silica gel using hexane–EtOAc gradient elution followed by HPLC using columns packed with Bondapak 10 CN and Spherisorb 5 CN.

Triol **1** had m.p. 108.0–110.5 °C. $[\alpha]_D$ +4.7° (*c* 0.43, $CHCl_3$). [Found: $(M-18)^{+\cdot}$ 306.2564. Calc. for $C_{20}H_{34}O_2$: 306.2559]. IR (CCl_4): 3300, 1660, 850 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.77 (s, H-20), 0.79 (s, H-19), 0.88 (s, H-18), 1.18 (d, *J* 0.9 Hz, H-17), 1.70 (dt, *J* 0.7 and 1.3 Hz, H-16), 3.86 (dd, *J* 2.9 and 9.8 Hz, H-12), 4.19 (dd, *J* 6.8 and −12.8 Hz, H-15a), 4.21 (dd, *J* 6.8 and −12.8 Hz, H-15b), 5.70 (tquintet, *J* 1.3 and 6.8 Hz, H-14). MS [*m/z* (%, composition)]: 306 (1, $M-18$), 291 (2, $C_{19}H_{31}O_2$), 288 (6, $C_{20}H_{32}O$), 275 (29, $C_{19}H_{31}O$), 255 (5, $C_{19}H_{27}$), 233 (3, $C_{16}H_{25}O$), 215 (4, $C_{16}H_{23}$), 205 (13, $C_{15}H_{25}$), 191 (100, $C_{14}H_{23}$ and $C_{13}H_{19}O$), 177 (32, $C_{13}H_{21}$ and $C_{12}H_{17}O$), 149 (20, $C_{11}H_{17}$ and $C_{10}H_{13}O$), 137 (28, $C_{10}H_{17}$ and $C_9H_{13}O$), 123 (36, C_9H_{15} and $C_8H_{11}O$), 109 (39, C_8H_{13} and C_7H_9O), 95 (49, C_7H_{11} and C_6H_7O), 81 (46, C_6H_9), 69 (43, C_5H_9 and C_4H_5O), 55 (53, C_4H_7 and C_3H_3O), 43 (59).

Compounds **2** and **3** had optical rotations and IR, ^1H NMR and mass spectra identical with those of the corresponding authentic samples.[7]

Compound **4** had m.p. 93.0–95.0 °C. $[\alpha]_D$ +11° (*c* 0.18, $CHCl_3$) [Found: $M^{+\cdot}$ 294.2294. Calc. for

$C_{18}H_{30}O_3$: 294.2195]. IR (CCl_4): 3613, 3487, 1709 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.83 (s, H-19), 0.88 (s, H-18), 0.98 (d, *J* 8.8 Hz, H-9), 1.06 (s, H-20), 1.25 (s, H-17), 2.17 (s, H-16), 3.02 (dd, *J* 2.1 and 8.8 Hz, H-11), 3.67 (d, *J* 2.1 Hz, H-12); MS [*m/z* (%, composition)]: 294 (7, *M*), 251 (12, $C_{16}H_{27}O_2$), 233 (3, $C_{16}H_{25}O$), 215 (3, $C_{16}H_{23}$), 189 (2, $C_{14}H_{21}$), 175 (6, $C_{13}H_{19}$ and $C_{10}H_{23}O_2$), 163 (10, $C_{12}H_{19}$ and $C_{11}H_{15}O$), 149 (10, $C_{10}H_{13}O$ and $C_{11}H_{17}$), 137 (8, $C_{10}H_{17}$ and $C_9H_{13}O$), 123 (12, C_9H_{15} and $C_8H_{11}O$), 109 (52, C_8H_{13} and C_7H_9O), 95 (30, C_7H_{11} and C_6H_7O), 81 (23, C_5H_5O), 69 (39, C_5H_9), 55 (24), 43 (100).

Compound **5** had $[\alpha]_D$ +28° (*c* 0.08, $CHCl_3$) [reported[11] $[\alpha]_D$ +64.5° (*c* 5, CCl_4)]; the IR, ^1H NMR and mass spectra were identical with those of an authentic sample.

Treatment of (12S,13E)-13-labdene-8,12,15-triol (**1**) *with acid.* (a) A solution of 16 mg of **1** in 1 ml of $CHCl_3$ was treated with 15 μl of stannic chloride at 0 °C for 30 min. Work-up and purification by HPLC (Spherisorb 5, hexane–EtOAc 80:20) gave 5.3 mg of (*E*)-8-hydroxy-13-labden-12-one (**11**), as an oil. $[\alpha]_D$ +21° (*c* 0.60, $CHCl_3$). IR ($CHCl_3$): 3590, 3480, 1665 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.79 (s, H-19), 0.83 (s, H-20), 0.87 (s, H-18), 1.13 (s, H-17), 1.79 (quintet, *J* 1.2 Hz, H-16), 1.87 (dq, *J* 1.2 and 7.0 Hz, H-15), 2.05 (dd, *J* 4.3 and 5.4 Hz, H-9), 2.61 (dd, *J* 5.4 and −17.5 Hz, H-11a), 2.83 (dd, *J* 4.3 and −17.5 Hz, H-11b), 6.82 (qq, *J* 1.2 and 7.0 Hz, H-14); MS [*m/z* (%)]: 306 (4, *M*), 288 (3), 273 (3), 245 (2), 221 (15), 195 (5), 191 (5), 177 (17), 137 (9), 121 (9), 111 (23), 95 (19), 83 (73), 69 (33), 55 (100), 43 (63).

(b) A solution of 21.7 mg of **1** in 5 ml of dioxane–water (3:1) and 200 μl of aqueous H_2SO_4 (5 %) was kept at room temperature and under nitrogen for 6 days. Work-up and separation by HPLC (Spherisorb 5; hexane–EtOAc 20:80) gave 4.8 mg of starting material (**1**) and 7.6 mg of (12*R*,13*E*)-8,12-epoxy-13-labden-15-ol (**2**), identical (optical rotation and IR, ^1H NMR and mass spectra) with an authentic sample.[7]

Epoxidation of (12S,13E)-13-labdene-8,12,15-triol (**1**). To a cold (0 °C) solution of 6.6 mg of **1** in 5 ml of $CHCl_3$ was added a solution of 10 mg of *m*-chloroperbenzoic acid in 1 ml of $CHCl_3$. After 2 h the reaction mixture was washed with aqueous $NaHCO_3$ and water, dried and concentrated. Purification by HPLC (Spherisorb 5 CN; EtOAc) gave 3.0 mg of (12*S*,13*R*,14*S*)-8,13-epoxylabdane-12,14,15-triol (**12**), identical (m.p., optical rotation, IR, ^1H and ^{13}C NMR and mass spectra) with an authentic sample.

Treatment of (12S,13S)-8,13-epoxy-14-labden-12-ol (**14**) *with osmium tetraoxide.* To a cold (0 °C) solution of 6.5 mg of **14** in 1 ml of pyridine was added dropwise a solution of 6.2 mg of OsO_4 in 1 ml of pyridine. After 1 h at 0 °C and 2 h at room temperature, a solution of 18 mg of $NaHSO_3$ in 4 ml of aqueous pyridine was added, and the mixture was stirred for 1.5 h. Dilution with water and extraction with EtOAc yielded, after separation by HPLC (Lichrosorb Diol 10; EtOAc), the (12*S*,13*R*,14*S*)- and (12*S*,13*R*,14*R*)-8,13-epoxylabdane-12,14,15-triols **12** and **13**.

Triol **12** (4.9 mg) had m.p. 190.0–192.5 °C. $[\alpha]_D$ +22° (*c* 0.30, $CHCl_3$). IR ($CHCl_3$): 3590 and 3455 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.77 (d, *J* 0.8 Hz, H-20), 0.80 (s, H-19), 0.86 (s, H-18), 1.21 (s, H-16), 1.28 (d, *J* 1.0 Hz, H-17), 3.7–3.9 (overlapping signals due to H-12, H-14 and H-15). MS [*m/z* (%)]: 279 (23, *M*−61), 261 (8), 243 (4), 192 (65), 191 (39), 177 (67), 149 (13), 137 (29), 123 (37), 109 (28), 95 (41), 81 (49), 69 (64), 55 (51), 43 (100).

Triol **13** (1.0 mg) was obtained as an oil. $[\alpha]_D$ +8.9° (*c* 0.09, $CHCl_3$). IR ($CHCl_3$): 3416 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.77 (s, H-20), 0.80 (s, H-19), 0.87 (s, H-18), 1.24 (s, H-16), 1.30 (d, *J* 0.8 Hz, H-17), 3.6–3.7 (overlapping signals due to H-14 and H-15a), 3.82 (dd, *J* 2.6 and 3.8 Hz, H-12), 3.96 (m, H-15b). MS [*m/z* (%)]: 279 (21, *M*−61), 261 (10), 243 (5), 192 (43), 191 (36), 177 (47), 149 (12), 137 (23), 123 (27), 109 (24), 95 (36), 81 (48), 69 (70), 55 (57), 43 (100).

Epoxidation of (11E)-8-hydroxy-14,15-dinor-11-labden-13-one (**15**). To a solution of 60 mg of **15** in 2.5 ml of methanol, kept at 0 °C, was added 0.2 ml of H_2O_2 (30 %) and 0.1 ml of aqueous NaOH (6M). The reaction mixture was stirred for 53 h at room temperature, diluted with water and extracted with EtOAc. The organic phase was washed with water, dried and concentrated. The residue was separated by HPLC (Spherisorb 5; hexane–EtOAc 80:20) to give 2.7 mg of 8-hydroxy-11-drimanal (**16**), identified by comparison of its ^1H NMR spectrum with that of an authentic

sample,[9] 10 mg (11*S*,12*R*)-11,12-epoxy-8-hydroxy-14,15-dinor-13-labanone (**4**), 15 mg of the corresponding 11*R*,12*S*-epimer **17**, and 6 mg of starting material (**15**).

(11*S*, 12*R*) - 11,12 - Epoxy - 8 - hydroxy - 14,15-dinor-13-labdanone had m.p., optical rotation, IR, ^{1}H NMR, ^{13}C NMR and mass spectra identical with those of the naturally occurring **4**.

17 had m.p. 84.0–86.5 °C. $[\alpha]_D$ −27° (*c* 0.97, $CHCl_3$). IR (CCl_4): 3569, 1714 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.82 (s, H-19), 0.88 (s, H-18), 1.00 (s, H-20), 1.18 (d, *J* 9.4 Hz, H-9), 1.47 (s, H-17), 2.13 (s, H-16), 3.19 (dd, *J* 2.2 and 9.4 Hz, H-11), 3.30 (d, *J* 2.2 Hz, H-12), 3.34 (s, OH). MS [*m/z* (%)]: 251 (3, *M*−43), 233 (10), 215 (12), 189 (11), 175 (16), 163 (28), 149 (9), 137 (8), 123 (11), 109 (21), 95 (24), 81 (22), 69 (37), 55 (25), 43 (100).

*Reduction of (11*E*)-8-hydroxy-14,15-dinor-11-labden-13-one* (**15**). To a solution of 69.9 mg of **15** in 5 ml of methanol were added 11.4 mg of $NaBH_4$. The reaction mixture was stirred at 10 °C for 2.5 h. Work-up and separation by HPLC (Spherisorb 5; hexane–EtOAc 15:85) gave 25.1 mg of (11*E*,13*R*)-14,15-dinor-11-labdene-8,13-diol (**18**) and 23.6 mg of the corresponding 13-epimer **19**.

18 had m.p. 138.5–139.5 °C. $[\alpha]_D$ +3.7° (*c* 2.2, $CHCl_3$). IR ($CHCl_3$): 3602, 3417 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.81 (s, H-19), 0.88 (s)/0.91 (s) (H-18/H-20), 1.19 (s, H-17), 1.30 (d, *J* 6.6 Hz, H-16), 4.35 (dq, *J* 4.3 and 6.6 Hz, H-13), 5.6–5.8 (overlapping signals due to H-11 and H-12), MS [*m/z* (%)]: 262 (7, *M*−18), 247 (4), 229 (4), 219 (3), 207 (5), 189 (4), 177 (11), 161 (8), 147 (6), 135 (23), 121 (30), 109 (49), 93 (62), 81 (30), 69 (40), 55 (32), 43 (100).

19 had m.p. 174.0–175.5 °C. $[\alpha]_D$ −1.1° (*c* 2.1, $CHCl_3$). IR ($CHCl_3$): 3590 and 3409 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.81 (s, H-19), 0.87 (s)/0.89 (s) (H-18/H-20), 1.20 (s, H-17), 1.28 (d, *J* 6.6 Hz, H-16), 4.32 (m, H-13), 5.5–5.7 (overlapping signals due to H-11 and H-12); MS [*m/z* (%)]: 262 (7, *M*−18), 247 (3), 229 (3), 219 (3), 207 (4), 189 (10), 177 (11), 161 (7), 147 (5), 135 (23), 121 (30), 109 (50), 93 (63), 81 (30), 69 (41), 55 (36), 43 (100).

*Epoxidation of the (11*E*)-14,15-dinor-11-labdene-8,13-diols* **18** *and* **19**. (a) To a solution of 9.3 mg of **18** and a catalytic amount of vanadyl acetylacetonate in 5 ml of benzene, kept at 0 °C, was added a solution of 10 µl of *tert*-butyl hydroperoxide in 0.5 ml of benzene. The reaction mixture was stirred at room temperature for 3 h, diluted with water and extracted with EtOAc. The organic phase was washed consecutively with a saturated aqueous solution of $FeSO_4$ and water and then dried. The solvent was removed under reduced pressure and the residue was purified by HPLC (Spherisorb 5; hexane–EtOAc 30:70) to give 6.7 mg of (11*R*,12*R*,13*R*)-11,12-epoxy-14,15-dinor-8,13-labdanediol (**20**), which had m.p. 123.0–124.0 °C; $[\alpha]_D$ +6.5° (*c* 0.51, $CHCl_3$). IR (CCl_4): 3505, 3410 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.83 (s, H-19), 0.88 (s, H-18), 1.02 (s, H-20), 1.19 (d, *J* 9.5 Hz, H-9), 1.30 (d, *J* 6.5 Hz, H-16), 1.44 (s, H-17), 2.89 (dd, *J* 2.5 and 2.9 Hz, H-12), 3.25 (dd, *J* 2.5 and 9.5 Hz, H-11), 4.01 (dq, *J* 2.9 and 6.5 Hz, H-13). MS [*m/z* (%)]: 263 (1, *M*−33), 251 (10), 233 (12), 215 (13), 195 (22), 177 (33), 163 (30), 149 (8), 137 (12), 123 (13), 109 (25), 95 (35), 81 (30), 71 (50), 55 (32), 43 (100).

Using the method described above, (a), the 8,13*S*-diol **19** (10.0 mg) was converted into (11*R*,12*R*,13*S*)-11,12-epoxy-14,15-dinor-8,13-labdanediol (**21**, 8.5 mg) which had m.p. 134.5–135.0 °C. $[\alpha]_D$ +22° (*c* 0.55, $CHCl_3$). IR (CCl_4): 3492, 3389 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.82 (s, H-19), 0.88 (s, H-18), 1.01 (s, H-20), 1.18 (d, *J* 9.4 Hz, H-9), 1.32 (d, *J* 6.5 Hz, H-16), 1.43 (s, H-17), 2.82 (dd, *J* 2.4 and 4.8 Hz, H-12), 3.16 (dd, *J* 2.4 and 9.4 Hz, H-11), 3.71 (dq, *J* 4.8 and 6.5 Hz, H-13); MS[*m/z* (%)]: 263 (1, *M*−33), 251 (7), 233 (12), 215 (13), 195 (20), 177 (31), 163 (29), 149 (7), 137 (12), 123 (13), 109 (26), 95 (35), 81 (30), 71 (50), 55 (33), 43 (100).

(b) To a solution of 9.8 mg of **18** in 5 ml of $CHCl_3$, kept at 0 °C, was added a solution of 7.8 mg of *m*-chloroperbenzoic acid in 1 ml of $CHCl_3$. After 3 h at room temperature, the reaction mixture was worked up in the usual manner and purified by HPLC (Spherisorb 5; hexane–EtOAc 30:70) to give 6.1 mg of **20**. Using method (b), **19** (9.8 mg) was converted into **21** (6.8 mg).

*Oxidation of the (11*R*,12*R*)-11,12-epoxy-14,15-dinor-8,13-labdanediols* **20** *and* **21**. A solution of 2.8 mg of **20** and 31 mg of pyridinium dichromate (PDC) in 1 ml of dimethylformamide was stirred at room temperature for 28 h. Work-up and chromatography over silica gel yielded 1.0 mg of **17** (IR, ^{1}H and ^{13}C NMR and mass spectra). The

same product (**17**, 0.8 mg) was obtained by treatment of **21** (2.1 mg) with PDC.

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References

1. Arndt, R., Wahlberg, I., Enzell, C. R. and Berg, J.-E. *Acta Chem. Scand., Ser. B 42* (1988) 294.
2. Wahlberg, I. and Enzell, C. R. *Nat. Prod. Rep. 4* (1987) 237.
3. Wahlberg, I., Karlsson, K., Curvall, M., Nishida, T. and Enzell, C. R. *Acta Chem. Scand., Ser. B 32* (1978) 203.
4. Nishida, T., Enzell, C. R. and Keeler, J. *J. Chem. Soc., Chem. Commun.* (1985) 1489.
5. Kato, T., Kanno, S., Tanemura, M., Kurozumi, A. and Kitahara, Y. *Bull. Chem. Soc. Jpn. 44* (1971) 3182.
6. Behr, D., Wahlberg, I., Nishida, T. and Enzell, C. R. *Acta Chem. Scand., Ser. B 31* (1977) 573.
7. Wahlberg, I., Nordfors, K., Nishida, T., Enzell, C. R. and Berg, J.-E. *Acta Chem. Scand., Ser. B 37* (1983) 209.
8. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. *Phytochemistry 18* (1979) 621.
9. Hlubucek, J. R., Aasen, A. J., Almqvist, S.-O. and Enzell, C. R. *Acta Chem. Scand., Ser. B 28* (1974) 131.
10. Berg, J.-E. *To be published.*
11. Vlad, P. F., Koltsa, M. N., Sibirtseva, V. E. and Kustova, S. D. *Zh. Obshch. Khim. 50* (1980) 195.
12. Reid, W. W. *Ann. Tab., Sect. 2* (1974) 145.
13. Aasen, A. J., Hlubucek, J. R. and Enzell, C. R. *Acta Chem. Scand., Ser. B 29* (1975) 589.
14. Demole, E. and Enggist, P. *Helv. Chim. Acta 61* (1978) 2318.
15. Wahlberg, I., Arndt, R., Nishida, T. and Enzell, C. R. *Acta Chem. Scand., Ser. B 40* (1986) 123.
16. Kimland, B., Aasen, A. J. and Enzell, C. R. *Acta Chem. Scand. 26* (1972) 2177.

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