Tobacco Chemistry. 57.* Two New Labdanic Compounds from Tobacco

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More than forty compounds belonging to the labdane/nor-labdane group have so far been encountered in tobacco.[2] However, as a result of genetic control, their presence is restricted to certain varieties such as Oriental and PB (Bergerac) tobaccos.[3,4] We now report the isolation of two new labdanic compounds (*1*,*2*) from a wax extract of green leaves of Greek tobacco.

Results. The first compound (*1*, $C_{19}H_{32}O_2$) contains an α,β-unsaturated aldehyde group (IR bands at 2710, 1685 and 1640 cm^{-1}), whose allocation to partial structure A (Fig. 1) was determined by proton spin decoupling and spin simulation experiments. Since the remaining oxygen was accommodated by a tertiary hydroxyl group (IR band at 3600 cm^{-1}; ^{13}C NMR singlet at δ 73.6, *cf.* Table 1) and since the ^{13}C NMR spectrum was devoid of signals due to additional sp^2 carbon atoms, it followed that aldehyde *1* is carbobicyclic.

A clue to its structure was provided by the ^{13}C NMR spectrum. Thus, since fourteen of the signals were of appropriate multiplicities and had chemical shift values close to those found for the C-1 to C-10 and C-17 to C-20 signals for (12*Z*)-abienol (*3*), aldehyde *1* was tentatively identified as 15-nor-8-hydroxy-12*E*-labden-14-al.

This assignment was verified by a direct comparison with an authentic sample, which was prepared by oxidative degradation of (12*E*)-abienol (*4*) using osmium tetroxide and sodium periodate.

The IR and ^{13}C NMR spectra demonstrated that the second compound (*2*, $C_{20}H_{34}O_2$) is a diol having a secondary and a tertiary hydroxyl group. It contains a conjugated diene system, which is arranged as shown in partial structure B (Fig. 1),[5] the *Z* geometry also following from a comparison of relevant ^{13}C NMR data with those of the (12*Z*)- and (12*E*)-abienols (*3*,*4*). Since the ^1H NMR spectrum also revealed the presence of four methyl groups attached to fully substituted sp^3 carbon atoms, it

Table 1. Carbon-13 chemical shifts and assignments for compounds *1*–*4*.[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
1	40.2	18.4	41.8	33.2	56.1	20.5	44.6	73.6	61.9	39.0	25.2	159.5	136.9	195.5		9.3	24.0	33.4	21.5	15.5
2	39.9	18.5	41.7	33.2	53.6	27.8	80.2	78.2	60.3	39.3	22.6	133.7	130.8	133.7	113.7	19.9	17.9	33.5	21.6	15.6
3	40.2	18.6	41.9	33.3	56.2	20.3	44.0	74.3	62.2	39.0	23.2	133.9	130.8	133.7	113.7	19.9	24.4	33.5	21.6	15.5
4	40.1	18.6	41.9	33.2	56.1	20.4	44.1	73.7	62.2	38.9	24.0	136.1	132.1	141.7	110.0	11.8	24.1	33.5	21.6	15.4

[a] δ-Values in $CDCl_3$ relative to TMS.

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* For part 56 see Ref. 1.

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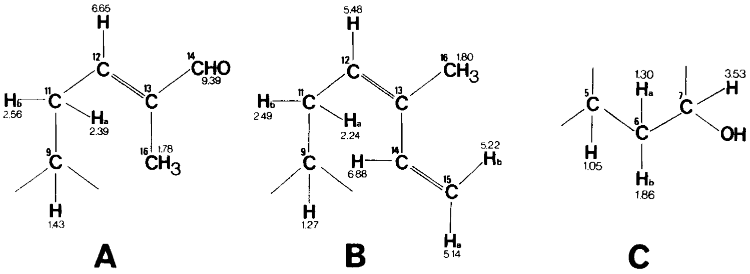


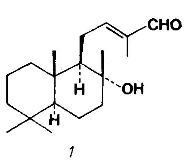
Fig. 1. $^1H-^1H$ coupling constants in Hz.
A. $J_{9,11a}=5.0$; $J_{9,11b}=5.5$; $J_{11a,11b}=-16$; $J_{11a,12}=6.5$; $J_{11b,12}=6.5$.
B. $J_{9,11a}=4.1$; $J_{9,11b}=5.7$; $J_{11a,11b}=-15.6$; $J_{11a,12}=6.4$; $J_{11a,16}=1.2$
$J_{11b,12}=7.9$; $J_{11b,16}=1.2$; $J_{12,14}=0.9$; $J_{12,15a}=1.5$; $J_{12,15b}=0.9$
$J_{12,16}=1.2$; $J_{14,15a}=10.4$; $J_{14,15b}=17.4$; $J_{15a,15b}=1.5$.
C. $J_{5,6a}=12.1$; $J_{5,6b}=1.4$; $J_{6a,6b}=-12.6$; $J_{6a,7}=11.7$; $J_{6b,7}=4.7$.

seemed most plausible that diol *2* is a diterpenoid of the labdane type.

This view was reinforced by the ^{13}C NMR data. Thus, eight signals in the spectrum of diol *2*, which are not associated with the side-chain, were virtually superimposable with those due to C-1 to C-4, C-10 and C-18 to C-20 for (12*Z*)-abienol (*3*), thereby showing that ring A is non-oxygenated and that the two hydroxyl groups are present in ring B. Their allocation to C-7 and C-8 followed from the observation that the C-6 to C-8 signals for diol *2* are downfield and the C-5, C-9 and C-17 signals are upfield from the corresponding signals for (12*Z*)-abienol (*3*), a result expected if a hydroxyl substituent is introduced at C-7 in (12*Z*)-abienol (*3*).[6]

In agreement with this assignment the mass spectrum of diol *2* contained diagnostically useful ions at *m/z* 207 and 150, which are analogous to the ions at *m/z* 191 and 134 in the spectrum of (12*Z*)-abienol (*3*)[7] and which are likely to be generated as shown in Scheme 1. Also the ^1H NMR spectrum displayed H-7 as a doublets of doublets at δ 3.53 (*cf.* partial structure C). Since the coupling constants of H-7 ($J=4.7$ and 11.7 Hz) are only consistent with a β-orientation of the hydroxyl group at C-7 and the shielding of C-17 with an *S*-configuration at C-8,[8] the new diol could be formulated as (7*S*,12*Z*)-12,14-labdadiene-7,8-diol (or enantiomer). The corresponding 12*E*-isomer, nidorellol (*5*) has previously been found in a *Nidorella* species.[9]

It may well be that 15-nor-8-hydroxy-12*E*-labden-14-al (*1*), the first C_{19} labdanic compound encountered in tobacco, is formed by oxidative biodegradation of (12*E*)-abienol (*4*).[10,11] However, (12*Z*)-abienol (*3*), which in contrast to its 12*E*-isomer (*4*)[4] is an abundant tobacco constituent and which can be converted to the majority of the tobacco labdanoids by oxidation of its side-chain,[12,13] is a more plausible precursor. This view is supported by the fact that treatment of (12*Z*)-abienol (*3*) with osmium tetroxide/sodium periodate yielded aldehyde *1*, the initially generated 15-nor-8-hydroxy-12*Z*-labden-14-al (*6*) evidently having undergone a facile isomerization.[14]



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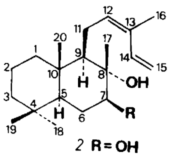

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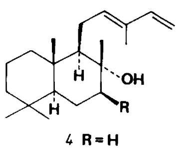

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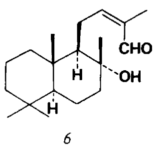

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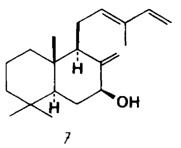

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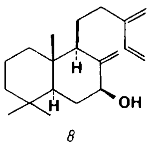

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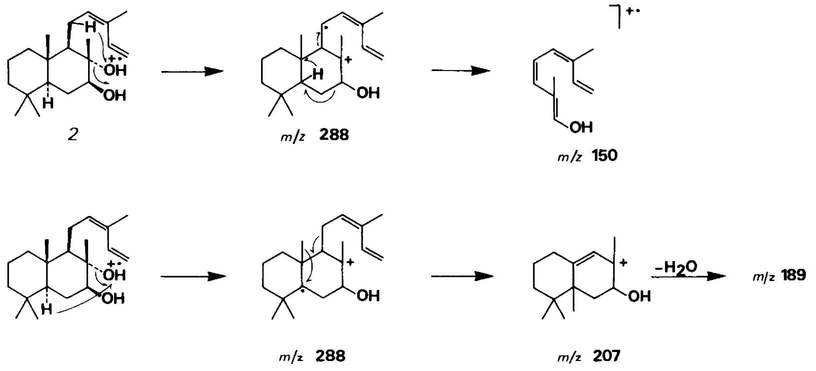

Scheme 1.

(7*S*,12*Z*)-12,14-Labdadiene-7,8-diol (*2*) is the first tobacco labdanoid encountered, which bears a substituent at C-7. Its generation may be explained by oxidation, *e.g.* microbial hydroxylation, of 12*Z*-abienol (*3*). It is of interest to note that (7*S*,12*E*)-8(17),12,14-labdatrien-7-ol (*7*) and (7*S*),8(17),-13(16),14-labdatrien-7-ol (*8*) have most recently been isolated from *Nicotiana raimondii.*[15]

Experimental. With the exception of accurate mass measurements, which were carried out on a Kratos' MS50-Stereo DS 50 SM/DS 50 S mass spectrometer/computer system and some of the ^1H NMR spectra, which were recorded on a Varian XL-200 spectrometer, the instruments specified in Ref. 16 were used.

Isolation. An extract (24 g) obtained by immersing green leaves of Greek *Nicotiana tabacum* (Basma Drama) in chloroform was distributed between hexane and methanol – water (80:20). The polar material obtained (16 g) was chromatographed over silica gel using a gradient of hexane – ethyl acetate as eluent to give fractions 1 (1 g), 2 (8 g) and 3 (6 g). Fraction 1 was a complex mixture, which was separated further by chromatography over silica gel and HPLC using columns packed with u-Porasil and u-Bondapak/CN to give 3.6 mg of 15-nor-8-hydroxy-12*E*-labden-14-al (*1*) and 10 mg of (7*S*,12*Z*)-12,14-labdadiene-7,8-diol (*2*).

15-Nor-8-hydroxy-12*E*-labden-14-al (*1*) was an oil and had $[\alpha]_D$ + 13° (*c* 0.30, $CHCl_3$) (Found: $M^{\cdot +}$ 292.2388. Calc. for $C_{19}H_{32}O_2$: mol. wt. 292.2402); IR (CCl_4) bands at 3600, 3440, 2710, 1685 and 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (s)/0.87 (s)/0.88 (s) (H-18/H-19/H-20) and 1.20 (s, H-17), for other data see Fig. 1. MS [*m/z* (%, composition)]: 292 (M, 3), 277 (18, $C_{18}H_{29}O_2$), 274 (17, $C_{19}H_{30}O$), 259 (8, $C_{18}H_{27}O$), 245 (4, $C_{17}H_{25}O$ and $C_{18}H_{29}$), 227 (3, $C_{17}H_{23}$), 216 (10, $C_{16}H_{24}$), 206 (21, $C_{15}H_{26}$), 191 (60, $C_{14}H_{23}$ and $C_{13}H_{19}O$), 177 (23, $C_{13}H_{21}$ and $C_{12}H_{17}O$), 163 (17, $C_{12}H_{19}$ and $C_{11}H_{15}O$), 150 (20, $C_{11}H_{18}$ and $C_{10}H_{14}O$), 137 (62, $C_{10}H_{17}$ and $C_9H_{13}O$), 123 (43, C_9H_{15} and $C_8H_{11}O$), 109 (77, C_8H_{13} and C_7H_9O), 95 (72, C_7H_{11} and C_6H_7O), 81 (63, C_6H_9 and C_5H_5O), 69 (84, C_5H_9 and C_4H_5O), 55 (72, C_4H_7 and C_3H_3O) and 43 (100, C_2H_3O and C_3H_7).

(7*S*,12*Z*)-12,14-Labdadiene-7,8-diol (or enantiomer) (*2*) was an oil, which decomposed on standing. It had $[\alpha]_D$ + 6.7° (*c* 0.30, $CHCl_3$) (Found: $M-18^{\cdot +}$ 288.2458. Calc. for $C_{20}H_{32}O$: 288.2453); IR ($CHCl_3$) bands at 3590 and 3400 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (s)/0.85 (s)/0.89 (s) (H-18/H-19/H-20) and 1.17 (s, H-17); MS [*m/z* (%, composition)]: 306 (M, 1), 288 (18), 273 (4, $C_{19}H_{29}O$), 270 (13, $C_{20}H_{30}$), 251 (12, $C_{16}H_{27}O_2$), 207 (21, $C_{14}H_{23}O$), 189 (18, $C_{14}H_{21}$), 177 (47, $C_{13}H_{21}$), 164 (22, $C_{11}H_{16}O$), 150 (63, $C_{10}H_{14}O$), 137 (33, $C_9H_{13}O$ and $C_{10}H_{17}$), 123 (77, C_9H_{15} and $C_8H_{11}O$), 109 (51, C_8H_{13} and C_7H_9O), 95 (54, C_7H_{11} and C_6H_7O), 81 (78, C_6H_9), 69 (78, C_5H_9 and C_4H_5O), 55 (54, C_4H_7 and C_3H_3O) and 43 (100).

*Conversion of the (12*E*)- and (12*Z*)-abienols (4, 3) to 15-nor-8-hydroxy-12*E*-labden-14-al (1).* A solution of 50 mg of (12*E*)-abienol (*4*) in 20 ml of dioxane – water (3:1) was stirred with 20 mg of osmium tetroxide at 0 °C for 5 min. After addition of 300 mg of sodium periodate the mixture was stirred at 0 °C for 1 h. Dilution with water, extraction with ether and chromatography over silica gel using a hexane – ethyl acetate gradient afforded 7.7 mg of 15-nor-8-hydroxy-12*E*-labden-14-al, which proved to be identical in all respects to the naturally occurring aldehyde (*1*).

Aldehyde *1* was also obtained by treatment of (12*Z*)-abienol (*3*) with osmium tetroxide/sodium periodate using the same conditions as those described above.

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