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# Tobacco Chemistry. 52. Seven New Nor-cembranoids Isolated from Greek Tobacco

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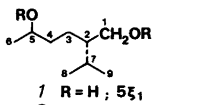
Seven new nor-cembranoids were isolated from Greek tobacco and shown to be (2*S*,5ξ)-2-isopropyl-1,5-hexanediol (*1*), (2*E*,6ξ)-3-isopropyl-2-heptene-1,6-diol (*2*), (2ξ,3*S**,4*S**,5*S**,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediol (*3*), the two (3*E*,5*S*)-1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-ones epimeric at C-2 (*4*, *5*), (3*R**,4*S**,5*S**,8ξ)-3,4-epoxy-5-isopropyl-2-methyl-2,8-nonanediol (*6*) and (3ξ,4*E*,6ξ)-1,3-dihydroxy-6-isopropyl-3-methyl-4-decen-9-one (*7*) by spectral and chemical methods. The biogenesis of these compounds is discussed.

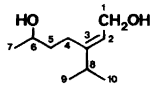
Previous studies have disclosed that the flavour fractions isolable from tobacco contain unusually large quantities of nor-isoprenoids, which are generated by biodegradation of diterpenoids, carotenoids and higher isoprenoids.[1] We now report the isolation and structure determination of seven new nor-cembranoids from Greek tobacco.

## RESULTS

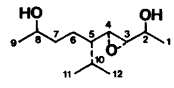
It followed from the IR (absorption at 3610 and 3410 $cm^{-1}$) and the $^{13}C$ NMR spectra (signals at $\delta$ 63.2 (t) and 68.6 (d), *cf.* Table 1) that the first tobacco isolate (*1*), $C_9H_{20}O_2$, was a diol having a primary and a secondary hydroxyl group. An analysis of the $^1H$ NMR spectra of *1* and the corresponding diacetate (*8*) established that the secondary hydroxyl group formed part of partial structure A and that the hydroxymethyl group was linked to a methine carbon atom.



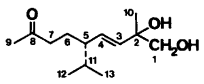

*1* R = H ; 5ξ1
*8* R = Ac ; 5ξ1
*10* R = H ; 5ξ2



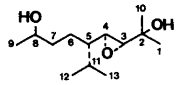

*2*



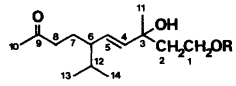

*3*, *14–16*



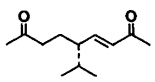

*4* 2ξ1
*5* 2ξ2



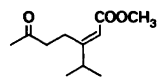

*6* 8ξ1
*24* 8ξ2



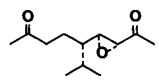

*7* R = H
*27* R = Ac



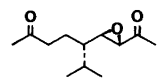
*9*



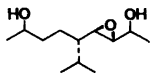

*11*



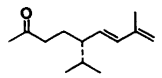
*12*



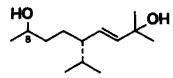
*13*



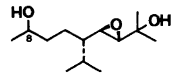

*17–20*



*21*




*22* 8ξ1
*23* 8ξ2




*25* 8ξ1
*26* 8ξ2

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*Table 1.* Carbon-13 chemical shifts and assignments for compounds *1*, *2*, *4*–*7*, *9*, *10*, *21*, *24* and *27*.[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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *1* | 63.2 | 46.7 | 24.3 | 37.3 | 68.6 | 23.6 | 28.5 | 19.91 | 19.7 | | | | | |
| *10* | 63.3 | 46.0 | 24.2 | 36.5 | 67.7 | 23.3 | 28.2 | 19.91 | 19.7 | | | | | |
| *2* | 58.4 | 121.3 | 149.8 | 25.6 | 38.2 | 66.9 | 23.5 | 33.4 | 22.31 | 21.8 | | | | |
| *4* | 70.1 | 73.2 | 136.2 | 130.8 | 48.9 | 26.2 | 42.1 | 210.5 | 29.8 | 24.3 | 32.0 | 20.61 | 19.2 | |
| *5* | 69.9 | 73.2 | 136.0 | 130.9 | 48.8 | 26.1 | 42.0 | 210.2 | 30.0 | 24.5 | 31.9 | 20.61 | 19.2 | |
| 6 | 25.1 | 67.9 | 65.2 | 58.7 | 46.6 | 26.5 | 36.7 | 68.1 | 23.5 | 27.9 | 29.7 | 20.51 | 19.5 | |
| *24*[b] | 25.1 | 67.9 | 65.0 | 58.7 | 46.7 | 26.3 | 36.7 | 68.4 | 23.6 | 27.9 | 29.7 | 20.51 | 19.5 | |
| *7* | 60.0 | 42.7 | 73.9 | 138.4 | 129.4 | 48.8 | 26.4 | 42.2 | 210.1 | 29.9 | 29.1 | 32.1 | 20.71 | 19.1 |
| *27*[c] | 61.5 | 40.8 | 72.0 | 138.1 | 129.8 | 48.7 | 26.2 | 42.1 | [d] | 30.0 | 29.0 | 32.1 | 20.71 | 19.1 |
| *9* | 27.0 | 198.1 | 132.6 | 149.8 | 49.1 | 25.3 | 41.6 | 208.1 | 30.0 | 31.9 | 20.61 | 19.3 | | |
| *21* | 114.7 | 141.8 | 132.2 | 134.6 | 49.6 | 26.4 | 41.9 | 208.1 | 29.9 | 18.8 | 32.5 | 20.31 | 19.3 | |

[a] $\delta$-Values in $CDCl_3$ relative to TMS. [b] Values obtained from the spectrum of a mixture of *3* and *24*. [c] $CH_3CO$ 21.0; $CH_3CO$ not visible. [d] Not visible.

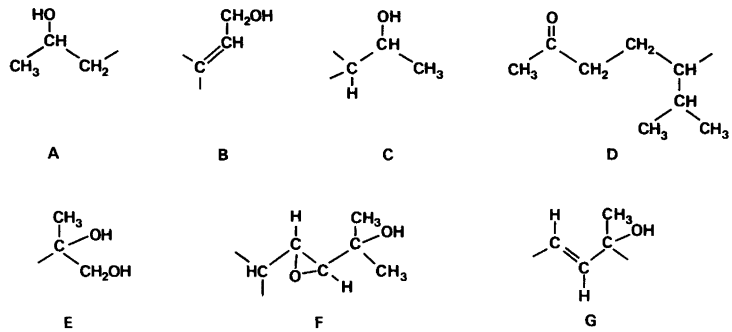
The remaining carbon atoms were present as an isopropyl substituent (six proton doublet $J = 6.5$ Hz at $\delta$ 0.92; IR bands at 1375 and 1390 $cm^{-1}$) and an $sp^3$ methylene group. Although these structural fragments may be linked in alternative ways, it seemed most likely from a biogenetic point of view that diol *1* was a 2-isopropyl-1,5-hexanediol.

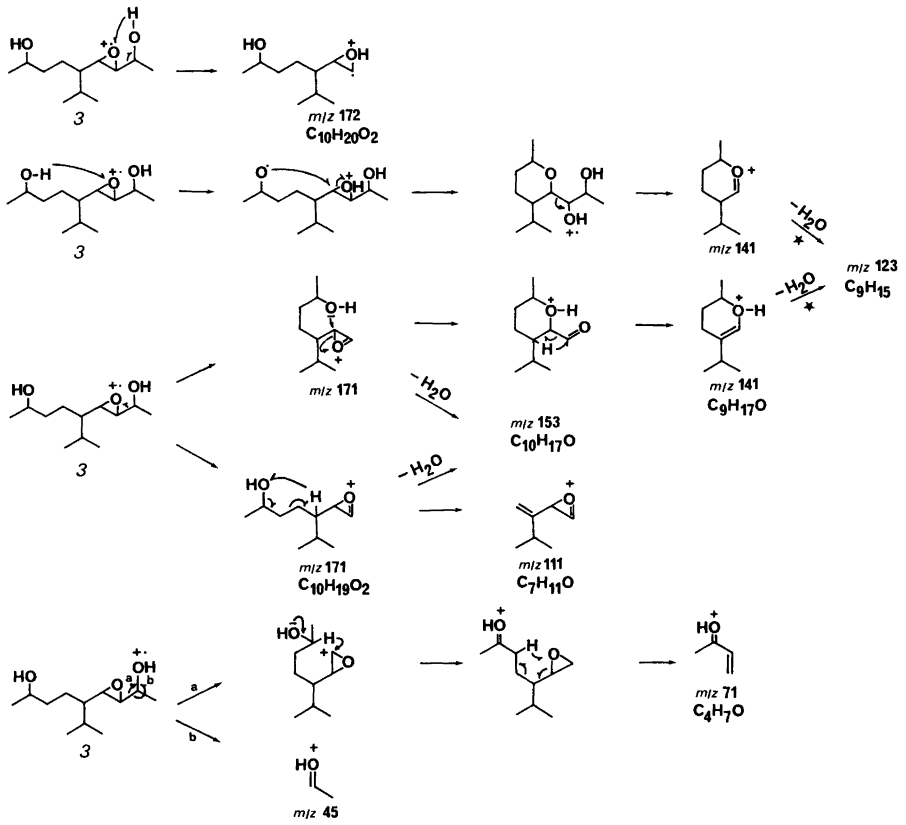
This assignment was verified by a synthesis, which involved ozonolysis of norsolanadione (*9*) followed by reductive work-up and which afforded the epimeric diols *1* and *10*. The most polar of these proved to be identical to the new tobacco diol, a result which settled the structure and the 2*S*-configuration but left the chirality at C-5 undetermined.

The second tobacco isolate (*2*), $C_{10}H_{20}O_2$, also contained a primary and a secondary hydroxyl group (*cf.* Table 1), which were allocated to partial structures B and A, respectively, by spin decoupling experiments. Since the $^1H$ and $^{13}C$ NMR showed that the remaining carbon atoms were present as an isopropyl and an $sp^3$ methylene group and since an NOE was registered between the methyl groups of the isopropyl group and the olefinic proton (7.5 %), diol *2* was provisionally identified as a 3-isopropyl-2*E*-heptene-1,6-diol.

Evidence, which verified this formulation but left the chirality at C-6 unsettled, was readily obtained by chemical means. Thus, reduction of methyl 3-isopropyl-6-oxo-2*E*-heptenoate (*11*)[2] using LAH yielded a diol, whose IR, $^1H$ NMR and mass spectra were identical to those of the new tobacco diol.

The third tobacco component (*3*), $C_{12}H_{24}O_3$, was obtained in a quantity too small to allow a complete spectral characterization. However, the $^1H$ NMR spectrum suggested the presence of end groups A and C, an isopropyl and an epoxide group, which may be combined to form a 3,4-epoxy-5-isopropyl-2,8-nonanediol. In agreement with this, the mass spectrum of *3* contained diagnostic peaks at *m/z* 172, 171, 141, 123, 111, 71 and 45, which can be explained






*Scheme 1.*

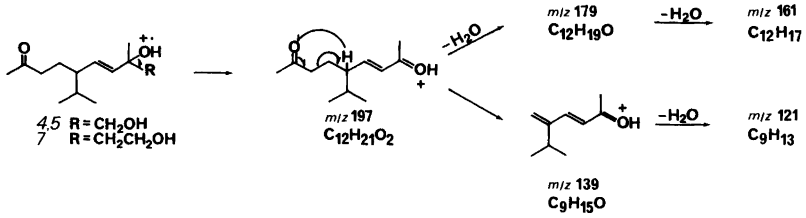
by the fragmentation reactions summarized in Scheme 1.

Confirmatory structural evidence was obtained by synthesis. Thus, epoxidation of norsolanadione (*9*) using hydrogen peroxide gave the (3*R*,4*S*,5*S*)- and (3*S*,4*R*,5*S*)-3,4-epoxy-5-isopropyl-2,8-nonanediones (*12*, *13*)[3] in the ratio 1:10. Treatment of the minor epoxydione *12* with $NaBH_4$ afforded, after separation by HPLC, two fraction $A_1$ and $A_2$ (ratio 2:1), each consisting of a 1:1 mixture of (3*S*,4*S*,5*S*)-3,4-epoxy-5-isopropyl-2,8-nonanediols (*3*, *14* and *15*, *16*). The reduction of the major epoxydione *13* proceeded in an analogous manner and yielded the four complementary (3*R*,4*R*,5*S*)-3,4-epoxy-5-isopropyl-5,8-nonanediols (*17* – *20*).

The $^1H$ NMR spectrum of the naturally occurring *3* proved to be identical to that recorded for the epoxydiols in fraction $A_1$. Since a detailed $^1H$ NMR analysis, which included spin decoupling experiments and LIS measurements carried out on the acetates obtained from the diols of fraction $A_1$ failed to provide further stereochemical information, it can only be concluded that *3* is a (2ξ,3*S**,4*S**,5*S**,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediol.

Compound *4*, $C_{13}H_{24}O_3$, contained a methyl ketone group (IR absorption at 1715 $cm^{-1}$; methyl singlet at $\delta$ 2.13), which was allocated to partial structure D by a comparison of the $^{13}C$ NMR spectrum with that of solanone (*21*). The remaining two oxygen atoms were accommodated by a primary and a tertiary hydroxyl group [OH-absorption in the IR spectrum; $^{13}C$ NMR signals at $\delta$ 70.1 (t) and 73.2 (s)]. The $^1H$ NMR spectrum allowed the placement of these in end group E.

Since the remaining two carbon atoms formed a disubstituted double bond, it seemed likely that *4* had a 1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-one structure. In harmony with this assignment




*Scheme 2.*

the mass spectrum of *4* displayed diagnostically useful peaks at *m/z* 197, 179, 161, 139 and 121, which are associated with the reactions outlined in Scheme 2.

The proposed structure of *4*, and also that of *5*, another constituent of Greek tobacco, which on account of its marked spectral similarities was deduced to be an epimer of *4*, was verified by chemical means. Thus, treatment of solanone (*21*) with osmium tetroxide furnished the expected (3*E*,5*S*)-1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-ones epimeric at C-2 in the ratio 1:2. These proved to be identical in all respects to compounds *5* and *4*, respectively, of Greek tobacco.

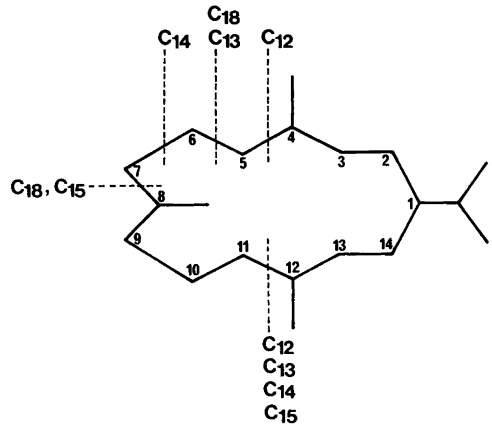
The $^1$H and $^{13}$C NMR spectra of compound *6*, $C_{13}H_{26}O_3$, indicated the presence of end group A, an isopropyl group, and a fully substituted carbon atom, which was linked to a hydroxyl group, two methyl groups and an *E*-disubstituted epoxide group, *i.e.* end group F. These groups and the remaining $sp^3$ methylene group may be combined to form a 3,4-epoxy-5-isopropyl-2-methyl-2,8-nonanediol. This assignment was in harmony with the mass spectral fragmentation of *6*, which resembled that of epoxydiol *3* and resulted in the formation of diagnostically useful ions of *m/z* 197, 179, 153, 141, 123, 111, 71 and 59 (*cf.* Scheme 1).

Conclusive evidence was obtained by a synthesis, which involved tosylation of a mixture of the two 1,2-dihydroxy-5*S*-isopropyl-2-methyl-3-nonen-8-ones (*4*, *5*) followed by reduction using LAH and separation by HPLC. The resultant fraction containing the 5-isopropyl-2-methyl-3-nonene-2,8-diols (*22*, *23*), although not separable by GC on a capillary column, was, as expected, a mixture of C-8 epimers. This conclusion was verified by the fact that subsequent epoxidation and separation yielded two fractions (ratio 1:3), each containing a 1:1 mixture of 3,4-epoxy-5*S*-isopropyl-2-methyl-2,8-nonanediols. The $^{13}$C NMR spectrum, showing doubling of the signals assigned to C-3, C-5, C-6, C-8 and C-9, indicated that the components of the major fraction had the same stereochemistry of the epoxide group but differed with respect to the configuration at C-8. Therefore and on the basis of previous findings reported by Demole and Demole,[3] the epoxydiols of the major fraction are provisionally assigned a (3*R*,4*S*,5*S*)-configuration (*6*, *24*) and those of the minor fraction a (3*S*,4*R*,5*S*)-configuration (*25*, *26*).

The epoxydiol isolated from tobacco (*6*), was found to be identical ($^1$H and $^{13}$C NMR) with one of the major epoxydiols. Hence, we propose that *6* is formulated as (3*R**,4*S**,5*S**,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediol.

Compound *7*, $C_{14}H_{26}O_3$, was a methyl ketone and incorporated end group D (*cf.* Table 1). Its tertiary hydroxyl group (OH-absorption in the IR spectrum of acetate *27*) had to be attached to the fully substituted carbon atom [$^{13}$C NMR signal at $\delta$ 73.9 (s)], which in turn was found to be linked to a methyl group and a disubstituted double bond of *E* configuration (ABX system with $J_{AB} = 16$ Hz in the $^1$H NMR spectrum of *27*). Thus, compound *7* contained end group G, which was linked *via* the double bond to end group D.

The remaining oxygen atom formed a primary hydroxyl group (two-proton doublet of doublets at $\delta$



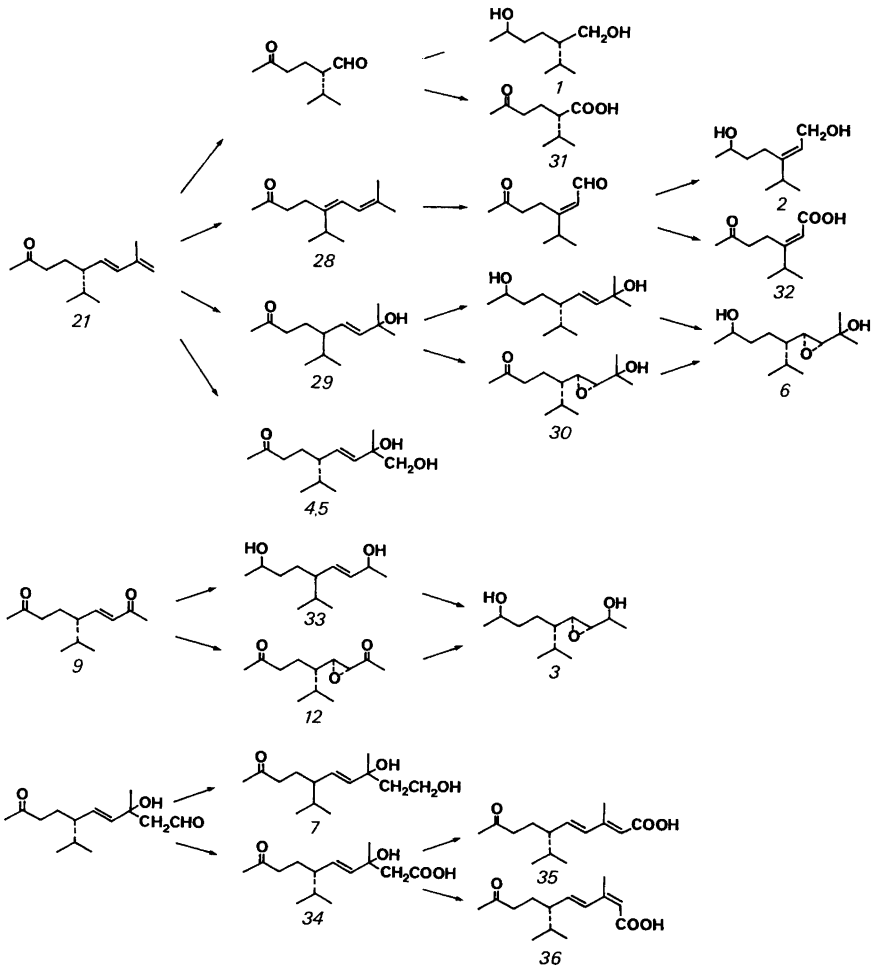

*Scheme 3.*

3.85, shifted to $\delta$ 4.21 in the $^1$H NMR spectrum of acetate *27*). Spin decoupling experiments demonstrated that this group was adjacent to a methylene group, which in turn was attached to the fully substituted carbon atom. In consistence with the formulation of 7 as a 1,3-dihydroxy-6-isopropyl-3-methyl-4-decen-9-one, its mass spectrum exhibited characteristic peaks at *m/z* 197, 179, 161, 139 and 121 (*cf.* Scheme 2). No assignment of stereochemistry of C-3 and C-6 has been made due to shortage of material.

## DISCUSSION

It is now generally agreed that the isopropyl-containing, irregular nor-isoprenoids isolable from the volatile fractions of many tobacco varieties arise by biodegradation of the cembranic diterpenoids present in the cuticular wax. These reactions may formally be depicted as involving initial cleavages of the parent cembrane as indicated in Scheme 3. The key metabolites thus generated, which have 18, 15, 14, 13 or 12 carbon atoms, then undergo further chemical alterations.[1] In harmony with this view, the 1*S*,2*E*-configurations of the cembranoid precursors have been encountered in all those nor-cembranoids, whose absolute configurations have been determined.[1]

The new compounds isolated from Greek tobacco (*1* – *7*) are readily incorporated into this biogenetic framework. Thus, as proposed in Scheme 4, solanone (*21*), a major $C_{13}$ nor-cembranoid,[4] may be a key metabolite in the formation of compounds *1* – *2* and *4* – *6*. This hypothesis is supported by the fact that the




*Scheme 4.*

assumed intermediates *28*–*30* as well as the oxo acids *31* and *32* have previously been found in tobacco.[2,3,5,6]

The biogenesis of compound *3* may involve norsolanadione (*9*)[7] as the key metabolite and proceed *via* the diol *33* or the epoxydione *12*, both of which are tobacco constituents.[3] Compound *7* represents an addition to the relatively few known $C_{14}$ nor-cembranoids, which include the acids *34*–*36*,[8-11] but as yet not the postulated key metabolite.

## EXPERIMENTAL

With the exception of accurate mass measurements, which were carried out on a Kratos' MS 50–Stereo DS 50 SM/DS 50S mass spectrometer/computer system, the instruments specified in Ref. 12 were used.

*Isolation.* (2*S*,5ξ)-2-Isopropyl-1,5-hexanediol (*1*, 6.5 mg), (2*E*,6ξ)-3-isopropyl-2-heptene-1,6-diol (*2*, 4.3 mg), (2ξ,3*S**,4*S**,5*S**,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediol (*3*, 0.4 mg), the (2$\xi_1$,3*E*,5*S*)- and (2$\xi_2$,3*E*,5*S*)-1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-ones (*4*, 12.1 mg; *5*, 9.7 mg), (3*R**,4*S**,5*S**,8ξ)-3,4-epoxy-5-isopropyl-2-methyl-2,8-nonanediol (*6*, 1.9 mg), and (3ξ,4*E*,6ξ)-1,3-dihydroxy-6-isopropyl-3-methyl-4-decen-9-one (*7*, 2.7 mg) were all isolated by liquid chromatography over silica gel and HPLC using columns packed with μ-Porasil, μ-Bondapak/CN and μ-Bondapak/C18 from fraction A3[13] of an extract obtained from 295 kg of sun-cured Greek tobacco.

(2*S*,5ξ)-2-Isopropyl-1,5-hexanediol (*1*) was obtained as an oil and had $[\alpha]_D$ −12° (*c* 0.05, $CHCl_3$) (Found: $[M-33]^+$ 127.1108. Calc. for $C_8H_{15}O$: 127.1123); IR ($CHCl_3$) bands at 3610, 3410, 1390 and 1375 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.92 (6H, d, *J* = 6.5 Hz), 1.22 (3H, d, *J* = 6 Hz), 3.63 (2H, m) and 3.79 (1H, broad m). Irradiation at the frequency of the methyl doublet at δ 1.22 affected the multiplet at δ 3.79, while conversely the methyl doublet at δ 1.22 collapsed to a singlet when the frequency of the multiplet at δ 3.79 was irradiated. The two-proton multiplet at δ 3,63 was converted to an AB system having *J* = 11 Hz on irradiation at the frequency of an obscured signal at δ 1.55; MS [*m/z* (%, composition)]: 127 (M − 33, 4), 112 (30, $C_8H_{16}$), 109 (26, $C_8H_{13}$), 97 (8, $C_6H_9O$), 83 (29, $C_6H_{11}$), 70 (59, $C_5H_{10}$), 69 (100, $C_5H_9$), 55 (66, $C_4H_7$) and 45 (48).

(2*E*,6ξ)-3-Isopropyl-2-heptene-1,6-diol (*2*) was an oil and had $[\alpha]_D$ −4° (*c* 0.4, $CHCl_3$) (Found: $M^{\cdot+}$ 172.1454. Calc. for $C_{10}H_{20}O_2$: 172.1463); IR ($CHCl_3$) bands at 3610, 3390 and 1660 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 1.04 (3H, d, *J* = 7 Hz), 1.05 (3H, d, *J* = 7 Hz), 1.22 (3H, d, *J* = 6.5 Hz), 3.78 (1H, six lines), 4.11 (1H, dd, *J* = 7 and 12 Hz), 4.25 (1H, dd, *J* = 7 and 12 Hz), and 5.52 (1H, broad t, *J* = 7 Hz). Irradiation at the frequency of the triplet at δ 5.52 converted the two doublets of doublets at δ 4.11 and 4.25 to an AB system with *J* = 12 Hz. The methyl doublet at δ 1.22 collapsed into a singlet when the frequency of the signal at δ 3.78 was irradiated, while conversely irradiation at the frequency of the methyl doublet at δ 1.22 converted the six line system at δ 3.78 to a triplet with *J* = 4 Hz. Saturation of the two methyl doublets at δ 1.05 produced a 7.5 % increase in the strength of the intensity of the olefinic signal at δ 5.52; MS [*m/z* (%, composition)]: 172 (M, 2), 154 (2, $C_{10}H_{18}O$), 139 (5, $C_9H_{15}O$), 136 (8, $C_{10}H_{16}$), 121 (15, $C_9H_{13}$), 111 (93, $C_7H_{11}O$), 95 (51, $C_7H_{11}$ and $C_6H_7O$), 83 (47, $C_6H_{11}$ and $C_5H_7O$), 67 (42), 55 (100) and 43 (91).

(2ξ,3*S**,4*S**,5*S**,8ξ)-3,4-Epoxy-5-isopropyl-2,8-nonanediol (*3*) (Found: $[M-33]^+$ 183.1344. Calc. for $C_{11}H_{19}O_2$: 183.1385) was obtained in a minute quantity, which made it impossible to record its optical rotation and its IR spectrum; $^1H$ NMR ($CDCl_3$): δ 0.97 (3H, d, *J* = 7 Hz), 0.98 (3H, d, *J* = 7 Hz), 1.23 (3H, d, *J* = 6 Hz), 1.29 (3H, d, *J* = 6.5 Hz), 2.86 (2H, m), 3.83 (1H, broad m) and 4.00 (1H, qd, *J* = 6.5 and 2.5 Hz); MS [*m/z* (%, composition)]: 183 (M − 33, 1), 172 (4), 171 (3, $C_{10}H_{19}O_2$), 153 (3), 141 (52, $C_9H_{17}O$), 123 (22, $C_9H_{15}$ and $C_8H_{11}O$), 111 (26, $C_7H_{11}O$ and $C_8H_{15}$), 99 (19, $C_6H_{11}O$ and $C_5H_7O_2$), 85 (34, $C_5H_9O$), 71 (62, $C_4H_7O$), 57 (46), 45 (38) and 43 (100).

(2$\xi_1$,3*E*,5*S*)-1,2-Dihydroxy-5-isopropyl-2-methyl-3-nonen-8-one (*4*) was an oil and had $[\alpha]_D$ −9° (*c* 1.2, $CHCl_3$) (Found: $[M-18]^{\cdot+}$ 210.1613. Calc. for $C_{13}H_{22}O_2$: 210.1620); IR ($CHCl_3$) bands at 3570, 3440 and 1715 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.85 (3H, d, *J* = 6.5 Hz), 0.89 (3H, d, *J* = 6.5 Hz), 1.27 (3H, s), 2.13 (3H, s), 3.42 (1H, d, *J* = 11 Hz), 3.52 (1H, d, *J* = 11 Hz) and 5.3 – 5.5 (2H, overlapping signals); MS [*m/z* (%, composition)]: 210 (M − 18, 1), 197 (5, $C_{12}H_{21}O_2$), 179 (4, $C_{12}H_{19}O$), 161 (5, $C_{12}H_{17}$), 139 (7, $C_9H_{15}O$), 121 (28, $C_9H_{13}$), 109 (29, $C_8H_{13}$ and $C_7H_9O$), 97 (15, $C_6H_9O$ and $C_7H_{13}$), 95 (14, $C_7H_{11}$ and $C_6H_7O$), 93 (13, $C_7H_9$), 81 (27), 71 (29), 55 (18) and 43 (100).

(2$\xi_2$,3*E*,5*S*)-1,2-Dihydroxy-5-isopropyl-2-methyl-3-nonen-8-one (*5*) was an oil and had $[\alpha]_D$ +0.9° (*c* 0.7, $CHCl_3$); IR ($CHCl_3$) bands at 3550, 3440 and 1715 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.84 (3H, d, *J* = 6.5 Hz), 0.89 (3H, d, *J* = 6.5 Hz), 1.29 (3H, s), 2.13 (3H, s), 3.43 (1H, d, *J* = 11 Hz), 3.52 (1H, d, *J* = 11 Hz), and 5.3 – 5.5 (2H, overlapping signals); MS [*m/z* (%)]: 210 (M − 18, 1), 197 (16), 179 (9), 161 (17), 139 (15), 121 (46), 109 (38), 97 (23), 95 (21), 93 (20), 81 (30), 71 (41), 55 (23) and 43 (100).

(3*R**,4*S**,5*S**,8ξ)-3,4-Epoxy-5-isopropyl-2-methyl-2,8-nonanediol (*6*) was isolated as an oil and had $[\alpha]_D$ +22° (*c* 0.2, $CHCl_3$) (Found: $[M-89]^+$ 141.1287. Calc. for $C_9H_{17}O$: 141.1279); IR ($CHCl_3$) bands at 3600, 3450, 1390 and 1375 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.96 (3H, d, *J* = 7 Hz), 0.97 (3H, d, *J* = 7 Hz), 1.22 (3H, d,

$J = 6$ Hz), 1.28 (3H, s), 1.32 (3H, s), 2.75 (1H, d, $J = 2.5$ Hz), 2.88 (1H, dd, $J = 2.5$ and 9 Hz) and 3.79 (1H, m, $W_{1/2} = 17$ Hz); MS [*m/z* (%, composition)]: 197 (M − 33, 3), 179 (2), 153 (3), 141 (52), 123 (25, $C_9H_{15}$), 111 (47, $C_7H_{11}O$ and $C_8H_{15}$), 83 (35, $C_6H_{11}$ and $C_5H_7O$), 71 (62, $C_4H_7O$), 59 (65) and 43 (100).

(3ξ,4*E*,6ξ)-1,3-Dihydroxy-6-isopropyl-3-methyl-4-decen-9-one (*7*) was an oil and had $[\alpha]_D$ −22° (*c* 0.3, $CHCl_3$) (Found: $[M-18]^{\cdot+}$ 224.1772. Calc. for $C_{14}H_{24}O_2$: 224.1776); IR ($CHCl_3$) bands at 3500 and 1715 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.84 (3H, d, $J = 6.5$ Hz), 0.89 (3H, d, $J = 6.5$ Hz), 1.34 (3H, s), 2.14 (3H, s), 3.85 (2H, dd, $J = 5$ and 6 Hz) and 5.3 − 5.6 (2H, overlapping signals). Irradiation at the frequency of the two-proton doublet of doublets at δ 3.85 affected a partially obscured signal at δ 1.8, while conversely, the signal at δ 3.85 was converted to a singlet on irradiation at δ 1.85. Irradiation at a slightly higher field, δ 1.7, converted the overlapping olefinic signals at δ 5.3 − 5.6 to an AB system with $J = 16$ Hz; MS [*m/z* (%, composition)]: 224 (M − 18, 2), 197 (2, $C_{12}H_{21}O_2$), 194 (3, $C_{13}H_{22}O$), 179 (2, $C_{12}H_{19}O$), 161 (5, $C_{12}H_{17}$), 151 (4, $C_{10}H_{15}O$), 139 (6, $C_9H_{15}O$), 136 (11, $C_{10}H_{16}$), 121 (25, $C_9H_{13}$ and $C_8H_9O$), 109 (19, $C_8H_{13}$ and $C_7H_9O$), 97 (26, $C_6H_9O$ and $C_7H_{13}$), 95 (16, $C_7H_{11}$ and $C_6H_7O$), 93 (26, $C_7H_9$), 83 (23), 71 (55), 55 (32) and 43 (100).

*Preparation of (2S,5ξ)-2-isopropyl-1,5-hexanediyl diacetate (8).* Acetylation using standard conditions converted diol *1* into (2*S*,5ξ)-2-isopropyl-1,5-hexanediyl diacetate (*8*), which was an oil and had $[\alpha]_D$ −1.4° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) bands at 1725 and 1255 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.89 (3H, d, $J = 7$ Hz), 0.90 (3H, d, $J = 6.5$ Hz), 1.22 (3H, d, $J = 6$ Hz), 2.04 (3H, s), 2.06 (3H, s), 3.48 (1H, dd, $J = 6$ and 13 Hz), 3.58 (1H, dd, $J = 5.5$ and 13 Hz) and 4.89 (1H, six line system); MS [*m/z* (%)]: 201 (M − 43, 1), 184 (1), 141 (13), 124 (35), 109 (31), 95 (12), 82 (31), 69 (41), 55 (18) and 43 (100).

*Preparation of the two (2S,5ξ)-2-isopropyl-1,5-hexanediols (1, 10).* A cold (−20 °C) solution of 26.3 mg of norsolanadione, (2*E*,5*S*)-5-isopropyl-3-nonene-2,8-dione (*9*), in 12 ml of MeOH was treated with ozone for 10 min. $NaBH_4$ (100 mg) was added and the mixture was left at room temperature for 1.5 h. Work up and HPLC using a column packed with μ-Porasil afforded as major products the 2*S*-isopropyl-1,5-hexanediols epimeric at C-5.

The least polar of these, *10*, 4.0 mg, was an oil and had $[\alpha]_D$ +11.3° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) bands at 3620, 3400, 1390 and 1375 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.92 (6H, d, $J = 6.5$ Hz), 1.22 (3H, d, $J = 6$ Hz), 3.63 (2H, m) and 3.81 (1H, m); MS [*m/z* (%)]: 127 (M − 33, 7), 112 (33), 109 (32), 97 (9), 83 (22), 70 (56), 69 (100), 55 (63) and 45 (45).

The most polar epimer, 5.8 mg, had $[\alpha]_D$ −16.4° (*c* 0.5, $CHCl_3$) and gave IR, mass, $^1H$ and $^{13}C$ NMR spectra identical to those of the tobacco diol *1*.

*Preparation of (±)-3-isopropyl-2E-heptene-1,6-diol (2).* A solution of 4.0 mg of methyl 3-isopropyl-6-oxo-2*E*-heptenoate (*11*)[2] in ether was reacted with an excess of LAH at room temperature for 1 h. Work up and chromatorgraphy over silica gel furnished 0.6 mg of (±)-3-isopropyl-2*E*-heptene-1,6-diol, whose IR, $^1H$ NMR and mass spectra were identical to those of the new tobacco diol *2*.

*Preparation of eight (2ξ,3ξ,4ξ,5S,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediols (3, 14 − 20).* To a solution of 65.6 mg of norsolanadione (*9*) in 10 ml of methanol, kept at 10 °C, was added 1.5 ml of 1 M NaOH and 0.75 ml of hydrogen peroxide (30 %). The reaction mixture was stirred for 2 h. Work up and separation by HPLC using a column packed with μ-Porasil and hexene/ethyl acetate (70:30) as solvent gave 2.6 mg of (3*R*,4*S*,5*S*)-3,4-epoxy-5-isopropyl-2,8-nonanedione (*12*) and 26.3 mg of the corresponding (3*S*,4*R*,5*S*)-isomer *13*.

*12* was an oil and had $[\alpha]_D$ −54° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) bands at 1710 and 1365 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 0.94 (3H, d, $J = 6.8$ Hz), 0.97 (3H, d, $J = 6.7$ Hz), 2.08 (3H, s), 2.17 (3H, s), 2.63 (2H, t, $J = 7.5$ Hz), 2.84 (1H, dd, $J = 2.2$ and 8.3 Hz) and 3.17 (1H, d, $J = 2.2$ Hz); MS [*m/z* (%)]: 169 (M − 33, 8), 149 (9), 135 (6), 123 (9), 109 (13), 97 (19), 81 (18), 69 (15), 55 (21) and 43 (100).

*13* was an oil and had $[\alpha]_D$ +31° (*c* 0.6, $CHCl_3$); IR ($CHCl_3$) bands at 1710 and 1365 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 1.00 (6H, d, $J = 6.7$ Hz), 2.08 (3H, s), 2.15 (3H, s), 2.47 (2H, t, $J = 7.5$ Hz), 2.91 (1H, dd, $J = 2.0$ and 8.3 Hz) and 3.15 (1H, d, $J = 2.0$ Hz); MS [*m/z* (%)]: 212 (M, 1), 194 (2), 169 (36), 151 (5), 139 (4), 123 (10), 109 (21), 99 (26), 81 (26), 69 (10), 55 (19) and 43 (100). The IR and $^1H$ NMR data for *12* and *13* agreed well with those published previously.[3]

To a solution of 3.0 mg of (3*R*,4*S*,5*S*)-3,4-epoxy-5-isopropyl-2,8-nonanedione (*12*) in 2 ml of methanol was added an excess of $NaBH_4$. The reaction mixture was stirred at 10 °C for 1.5 h. Work up and separation by HPLC using a column packed with μ-Porasil gave two fractions, $A_1$ and $A_2$.

The least polar fraction, $A_1$ 0.6 mg, was a 1:1 mixture of (2ξ,3*S*,4*S*,5*S*,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediols (*3*, *14*), which had IR ($CHCl_3$) bands at 3620, 3470, 1395 and 1375 $cm^{-1}$; the $^1H$ NMR and mass spectra were identical to those obtained for tobacco constituent *3*.

The most polar fraction, $A_2$ 0.3 mg, was also a 1:1 mixture of (2ξ,3*S*,4*S*,5*S*,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediols (*15*, *16*), which had $^1H$ NMR ($CDCl_3$): δ 0.95 (3H, d, $J = 7$ Hz), 0.97 (3H, d, $J = 7$ Hz), 1.22 (3H, d, $J = 6$ Hz), 1.32 (3H, d, $J = 6.5$ Hz), 2.78 (2H, overlapping signals) and 3.75 (2H, overlapping m); MS [*m/z* (%)]: 183 (M − 33, 2), 172 (1), 171 (2), 153 (6), 141 (98), 123 (42), 111 (33), 99 (28), 85 (47), 71 (94), 55 (71), 45 (37) and 43 (100).

Treatment of 3.5 mg of (3*S*,4*R*,5*S*)-3,4-epoxy-5-isopropyl-2,8-nonanedione (*13*) with an excess of

$NaBH_4$, work up and separation by HPLC over μ-Porasil yielded three fractions $B_1$, $B_2$ and $B_3$.

Fraction $B_1$, 1.0 mg, was a 1:1 mixture of two (2ξ,3*R*,4*R*,5*S*,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediols (*17*,*18*), which had $^1$H NMR ($CDCl_3$): δ 0.98 (6H, d, *J* = 7 Hz), 1.21 (3H, d, *J* = 6 Hz), 1.31 (3H, d, *J* = 6.5 Hz), 2.80 (2H, overlapping signals) and 3.73 (2H, overlapping m); MS [*m*/*z* (%)]: 172 (M − 44, 1), 171 (1), 153 (2), 141 (72), 123 (28), 111 (30), 95 (15), 85 (57), 71 (77), 55 (68), 45 (60) and 43 (100).

Fraction $B_2$, 0.4 mg, consisted of one (2ξ,3*R*,4*R*,5*S*,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediol (*19*), which had $^1$H NMR ($CDCl_3$): δ 0.98 (6H, d, *J* = 7 Hz), 1.21 (3H, d, *J* = 6 Hz), 1.29 (3H, d, *J* = 6.5 Hz), 2.83 (2H, overlapping signals) and 3.86 (2H, overlapping m); MS [*m*/*z* (%)]: 172 (M − 44, 1), 171 (2), 153 (2), 141 (100), 123 (41), 111 (38), 97 (18), 85 (59), 71 (91), 55 (61), 45 (50) and 43 (97).

Fraction $B_3$, 0.8 mg, consisted of one (2ξ,3*R*,4*R*,5*S*,8ξ)-3,4-epoxy-5-isopropyl-2,8-nonanediol (*20*), which had $^1$H NMR ($CDCl_3$): δ 0.98 (6H, d, *J* = 6.8 Hz), 1.21 (3H, d, *J* = 6.2 Hz), 1.30 (3H, d, *J* = 6.4 Hz), 2.77 (1 H, dd, *J* = 2.5 and 4 Hz), 2.87 (1H, dd, *J* = 2.5 and 8 Hz), 3.72 (1H, broad m) and 3.90 (1H, qd, *J* = 4 and 6.4 Hz); MS [*m*/*z* (%)]: 172 (M − 44, 6), 171 (8), 153 (2), 141 (56), 123 (21), 111 (32), 97 (19), 85 (30), 71 (58), 55 (56), 45 (29) and 43 (100).

*Preparation of the two (2ξ,3E,5S)-1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-ones (4, 5).* To a cooled (0 °C) solution of 52.2 mg of solanone (3*E*,5*S*)-5-isopropyl-2-methyl-1,3-nonadien-8-one (*21*) in 1 ml of pyridine was added dropwise a solution of 68.4 mg of osmium tetroxide in 1 ml of pyridine. After stirring at room temperature for 1 h, a solution of 126 mg of sodium bisulfite in aqueous pyridine was added. The reaction mixture was diluted with water, extracted with ether, washed with aqueous HCl, dried and evaporated. Column chromatography of the residue over silica gel and subsequent HPLC using a column packed with μ-Bondapak/$C_{18}$ furnished 10.1 mg of (2ξ$_2$,3*E*,5*S*)-1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-one and 21.7 mg of (2ξ$_1$,3*E*,5*S*)-1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-one, which proved to be identical ($[\alpha]_D$, IR, $^1$H NMR) to compounds *5* and *4*, respectively, of Greek tobacco.

*Preparation of the four (3ξ,4ξ,5S,8ξ)-3,4-epoxy-5-isopropyl-2-methyl-2,8-nonanediols (6, 24 − 26).* To a solution of a mixture of the (3*E*,5*S*)-1,2-dihydroxy-5-isopropyl-2-methyl-3-nonen-8-ones (*4*, *5*) in 0.5 ml of pyridine, kept at 0 °C, was added dropwise a solution of 65 mg of *p*-toluene sulfonyl chloride in 1 ml of pyridine. The reaction mixture was left at room temperature for 17 h and worked up.

Without further purification the tosylates obtained were reacted with an excess of LAH in 3 ml of ether at room temperature for 0.5 h. Work up and HPLC using a column packed with μ-Porasil furnished 2.3 mg of a mixture consisting of the two 5*S*-isopropyl-2-methyl-3-nonene-2,8-diols (*22*, *23*), which had $^1$H NMR ($CDCl_3$): δ 0.82 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 1.18 (3H, d, *J* = 6 Hz), 1.33 (6H, s), 3.75 (1H, broad m), 5.35 (1H, dd, *J* = 8 and 15.5 Hz) and 5.59 (1H, d, *J* = 15.5 Hz); MS [*m*/*z* (%)]: 196 (M − 18, 3), 181 (9), 163 (5), 153 (7), 135 (5), 123 (16), 109 (18), 95 (17), 81 (29), 69 (35), 59 (11), 55 (16) and 43 (100).

To a solution of 2.3 mg of the mixture of *22* and *23* and 5 mg of sodium acetate in 0.5 ml of $CHCl_3$ was added a solution of 2.2 mg of *m*-chloroperbenzoic acid in 1 ml of $CHCl_3$. The reaction mixture was kept at room temperature for 6 h. Work up and HPLC using a column packed with μ-Bondapak/CN gave two fractions.

The least polar fraction, 0.3 mg, contained a mixture of (3*S*,4*R*,5*S*)-3,4-epoxy-5-isopropyl-2-methyl-2,8-nonanediols (*25*, *26*) and had $^1$H NMR ($CDCl_3$): δ 0.99 (6H, d, *J* = 7 Hz), 1.21 (3H, d, *J* = 6 Hz), 1.28 (3H, s), 1.32 (3H, s), 2.72/2.73 (1H, d, *J* = 2.5 Hz), 2.92 (1H, dd, *J* = 2.5 and 8 Hz) and 3.79 (1H, broad m); MS [*m*/*z* (%)]: 197 (M − 33, 0.5), 179 (0.5), 141 (54), 123 (22), 111 (61), 93 (22), 85 (41), 71 (60), 59 (53) and 43 (100).

The most polar fraction, 0.8 mg, containing a mixture of (3*R*,4*S*,5*S*)-3,4-epoxy-5-isopropyl-2-methyl-2,8-nonanediols (*6*, *24*) gave a $^1$H NMR spectrum, which with exception of the presence of a one-proton doublet at δ 2.74 (*J* = 2.5 Hz) assigned to H-3 of *24*, was identical to that obtained for compound *6* isolated from Greek tobacco. The mass spectrum recorded for the synthetic mixture (*6*, *24*) was indistinguishable from that of the naturally occurring compound (*6*).

*Preparation of (3ξ,4E,6ξ)-1-acetoxy-3-hydroxy-6-isopropyl-3-methyl-4-decen-9-one (27).* Acetylation using standard conditions converted *7* into (3ξ,4*E*,6ξ)-1-acetoxy-3-hydroxy-6-isopropyl-3-methyl-4-decen-9-one (*27*), which had IR ($CHCl_3$) bands at 3590, 3460, 1735, 1715, 1245 and 1220 $cm^{-1}$; $^1$H NMR ($CDCl_3$): δ 0.83 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.5 Hz), 1.32 (3H, s), 1.89 (2H, m), 2.05 (3H, s), 2.13 (3H, s), 2.36 (2H, m), 4.21 (2H, t, *J* = 7 Hz), 5.37 (1H, dd, *J* = 6 and 16 Hz) and 5.51 (1H, d, *J* = 16 Hz). Irradiation at the frequency of the triplet at δ 4.21 converted the multiplet at δ 1.89 to a broad singlet. Conversely, the triplet at δ 4.21 collapsed to a singlet when the frequency of the signal at δ 1.89 was irradiated; MS [*m*/*z* (%)]: 206 (M − 18 − 60, 7), 194 (3), 163 (8), 148 (13), 136 (14), 121 (22), 105 (36), 93 (44), 79 (12), 71 (11), 55 (12) and 43 (100).

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