

Tobacco Chemistry. 67.* Two New 20-Norcembranoids from Tobacco

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Two new C-20 norcembranoids have been isolated from flowers of Greek tobacco. They have been identified as (1*S*,2*E*,4*S*,6*R*,7*E*)-4,6-dihydroxy-20-nor-2,7-cembradien-12-one (**1**) and (1*S*,2*E*,4*S*,6*R*,7*R*,8*R*)-4,6-dihydroxy-7,8-epoxy-20-nor-2-cembren-12-one (**2**) by spectral and chemical methods. The conformation about the 5,6 bond in **1** and **2** is discussed, as are plausible biogenetic pathways.

The cuticular wax of the leaf and flower of most tobacco varieties contains substantial amounts of diterpenoids of the cembrane class. (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-Cembratriene-4,6-diol (**3**) and the corresponding 4-epimer (**4**) are the major components and, as indicated by results of biomimetic experiments, the principal precursors of the majority of the other tobacco cembranoids. The metabolism of the cembranoids also involves breakage of carbon-carbon bonds in the macrocyclic ring, resulting in the formation of carbocyclic compounds having 18 to 12 carbon atoms.² We now report the isolation from flowers of Greek tobacco of two 20-norcembranoids (**1**, **2**), the first C₁₉ compounds.

Results

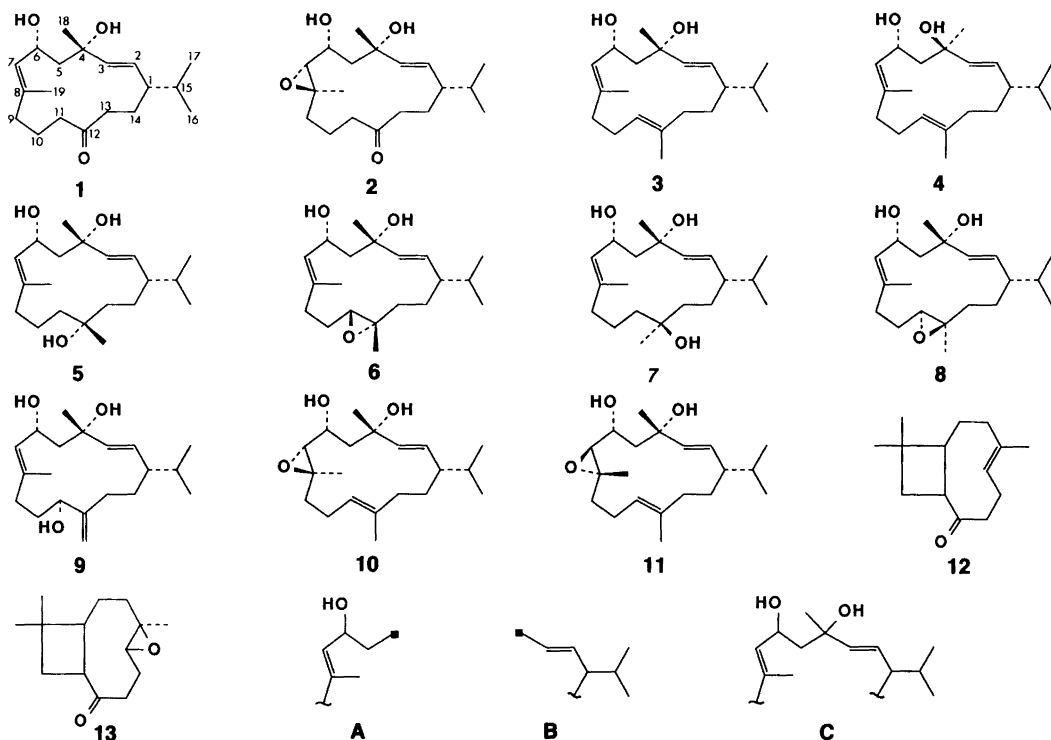
The first new compound (**1**), C₁₉H₃₂O₃, is a diol (IR: 3602 and 3487 cm⁻¹) having a secondary and a tertiary hydroxyl group [¹³C NMR: δ 68.1 (d) and 74.1 (s)]. The remaining oxygen atom is accommodated by a carbonyl group [IR: 1709 cm⁻¹; ¹³C NMR: δ 211.7 (s)]. Since the ¹H and ¹³C NMR spectra are also consistent with the presence of two double bonds, of which one is tri- and one is *E*-disubstituted (*J* = 15.3 Hz), it follows that diol **1** is carbomonocyclic.

The occurrence of four methyl groups, of which two form part of an isopropyl group, one is attached to the carbon carrying the tertiary hydroxyl group and one is vinylic, shows that the carbomonocyclic ring in **1** is fourteen-membered.

¹H–¹H and ¹H–¹³C chemical shift correlation spectroscopy³⁻⁵ was used to formulate partial structures **A** and **B**. These were combined to **C** via the fully substituted carbon atom bearing the tertiary hydroxyl group. Although there are alternative ways to include the carbonyl group and the remaining five *sp*³ methylene groups in the carbocyclic ring, it seems most plausible from a biogenetic point of view that **1** is a 4,6-dihydroxy-20-nor-2,7-cembradien-12-one. This assignment was reinforced by treatment of **1** with methyl-lithium in tetrahydrofuran. Two products were obtained: the major one proved to be identical in all respects to (1*S*,2*E*,4*S*,6*R*,7*E*,12*R*)-2,7-cembradiene-4,6,12-triol (**5**), obtained by LAH-reduction of the 11*S*,12*S*-epoxide **6**.⁶ The minor product was identified as the corresponding 12*S*-epimer **7** by comparison with an authentic sample obtained from the 11*R*,12*R*-epoxide **8**⁶ by reduction using LAH. These results allowed an unequivocal identification of **1** as (1*S*,2*E*,4*S*,6*R*,7*E*)-4,6-dihydroxy-20-nor-2,7-cembradien-12-one.

With the structure and stereochemistry at hand, it can be concluded from the ¹³C NMR

*For Part 66, see Ref. 1.



results listed in Table 1 and from the ^1H NMR results listed in Table 2 that the conformation about the 5,6 bond in **1** is reminiscent of that of the 4*S*, 6*R*, 11*S*-triol **9**^{7,8} but different from that of the 4*S*, 6*R*-diol **3**.⁸ Moreover, the conversion of **1** to the 4*S*, 6*R*, 12*R*- and 4*S*, 6*R*, 12*S*-triols **5** and **7** evidently occurs with retention of the conformation.

The spectral data indicated that the second new compound (**2**) is structurally closely related to **1**. Thus, its ^1H NMR spectrum retains the signals due to the isopropyl group, the proton under the secondary hydroxyl group (H-6), the 2,3 double bond and the methyl group attached to C-4 (H-18). The signals arising from the vinylic methyl group (H-19) and from H-7 are replaced, however, by a methyl singlet at δ 1.28 and a one-proton doublet at δ 2.91. These results, together with the elemental composition, $\text{C}_{19}\text{H}_{32}\text{O}_4$, and the presence of a carbonyl band at 1711 cm^{-1} in the IR spectrum suggested that **2** is a 4,6-dihydroxy-7,8-epoxy-20-nor-2-cembrene-12-one. This assignment was readily verified and a 1*S*, 2*E*, 4*S*, 6*R*-stereochemistry was established by

treatment of **1** with *m*-chloroperbenzoic acid. The reaction proceeded with a highly stereoselective attack on the 7,8 double bond, the resultant 7,8-epoxide having spectral data and optical rotation in good agreement with those of the naturally occurring **2**.

Information on the stereochemistry of the epoxide group in **2** was provided by a comparison of its ^{13}C NMR spectrum with those of the (7*R*, 8*R*)- and (7*S*, 8*S*)-epoxides **10** and **11**.⁹ It can be seen in Table 1 that the chemical shift values for C-2 to C-8, C-18 and C-19 of **2** are close to the corresponding data for **10** but sufficiently different from the data for **11** to conclude that **2** is (1*S*, 2*E*, 4*S*, 6*R*, 7*R*, 8*R*)-4,6-dihydroxy-7,8-epoxy-20-nor-2-cembrene-12-one. The ^1H NMR chemical shift values and coupling constants listed in Table 2 support this assignment and suggest that **2** and **10** are conformationally similar with respect to their C-4 to C-8 portions.⁹

The two new compounds (**1**, **2**) are formally derived from an appropriate parent cembranoid by loss of C-20. They are hence additions to the oxo-containing norterpeneoids previously found in

Table 1. ^{13}C NMR chemical shift values and assignments for compounds 1, 2, 5, 7 and 9–11.^a

Compound	Carbon																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	47.4	127.9	140.2	74.1	46.3	68.1	129.3	137.5	36.9	20.2	39.8	211.7	40.6	26.4	32.8	19.2	20.8	32.1	15.4	
2	46.1	128.6	138.7	75.1	42.1	71.0	63.4	61.4	35.4	18.8	38.0	210.1	39.4	25.4	32.9	19.1	20.7	32.6	15.0	
5	50.7	129.0	138.0	73.6 ^b	46.6	68.0	127.1	138.5	38.1	21.7	39.8 ^c	73.2 ^b	40.7 ^c	26.5	32.1	19.6	21.0	31.9	17.1	27.6
7	50.9	129.3	137.9	73.6 ^b	46.9	68.3	126.4	138.4	37.8	20.5	39.9 ^c	73.7 ^b	40.0 ^c	27.8	31.9	19.7	21.0	31.9	17.5	28.4
9	49.0	127.4	139.8	74.1	46.5	68.5	128.5	137.8	29.8	32.9	74.8	151.6	34.6	29.3	32.2	19.2	20.8	33.4	16.0	111.2
10	43.7	128.9	138.6	74.8	42.6	71.9	63.4	61.1	39.8	23.6	125.1	133.5	36.6	28.1	34.1	19.2	19.9	32.3	15.7	13.7
11	46.8	129.0	137.5	72.4	49.5	67.2	68.2	63.2	36.7	22.5	124.1	135.0	36.4	28.1	32.7	19.7	20.7	29.1	17.9	14.7

^a φ -Values in CDCl_3 relative to TMS. ^{b,c} Assignment may be reversed.Table 2. Chemical shift values (δ) and coupling constants (J in Hz) for the H-5a, H-5b, H-6 and H-7 signals in the ^1H NMR spectra of compounds 1–3, 5, 7, 9 and 10.

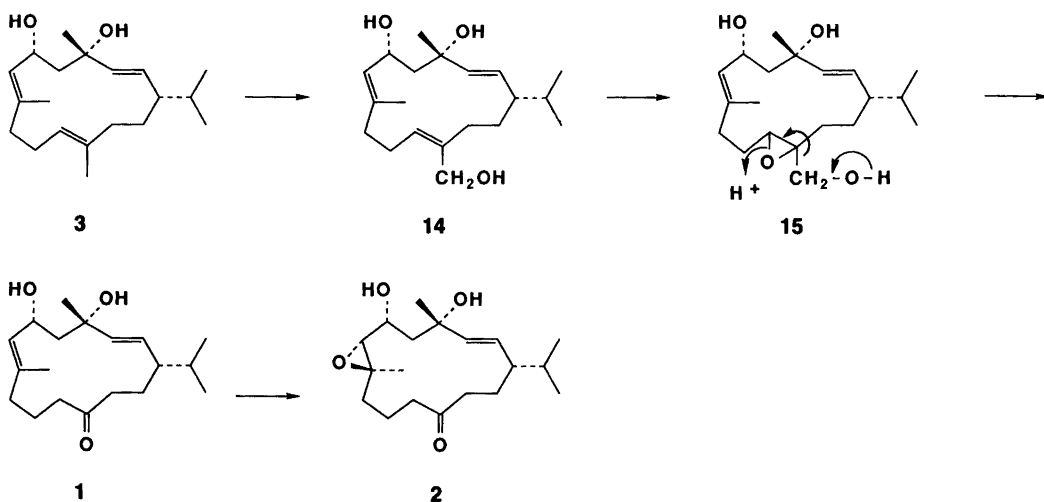
Compound	H-5a	H-5b	H-6	H-7
1	1.79 $J_{5a,6}=1.5$	2.21 $J_{5b,6}=6.4$	4.70 $J_{6,7}=9.1$	5.64
3	1.96 $J_{5a,6}=7.9$	2.03 $J_{5b,6}=2.9$	4.49 $J_{6,7}=9.2$	5.34
9	1.80 $J_{5a,6}=2.7$	2.22 $J_{5b,6}=5.5$	4.67 $J_{6,7}=9.1$	5.59
5	1.79 $J_{5a,6}=2.0$	2.19 $J_{5b,6}=6.5$	4.69 $J_{6,7}=8.7$	5.73
7	1.79 $J_{5a,6}=2.2$	2.19 $J_{5b,6}=6.7$	4.72 $J_{6,7}=9.1$	5.67
2	1.99 $J_{5a,6}=0.9$	2.23 $J_{5b,6}=6.7$	3.65 $J_{6,7}=9.7$	2.91
10	1.97 $J_{5a,6}=1.0$	2.25 $J_{5b,6}=6.8$	3.75 $J_{6,7}=9.2$	3.37

nature, e.g. 12-norcaryophyllen-2-one (12) and the corresponding epoxide, kobusone (13), of lavender oil.¹⁰ One possible route to 1 and 2, outlined in Scheme 1, would involve the 4*S*,6*R*,20-triol 14, a recently discovered tobacco constituent,¹¹ and a corresponding 11,12-epoxide (15) as intermediates. The latter undergoes an acid-induced rearrangement leading to the prerequisite loss of C-20 and the formation of 1. Subsequent oxidation of 1 gives the 7*R*,8*R*-epoxide 2.

Experimental

With the exception of optical rotations, which were recorded on a Perkin-Elmer 241 polarimeter, the instruments specified in Ref. 12 were used.

Isolation. A chloroform extract (83 g) of flowers of *Nicotiana tabacum* (Basma) was separated into five fractions, A (12.7 g), B (4.7 g), C (8.0 g), D (30 g), and E (3.6 g), by flash chromatography (silica gel; hexane/EtOAc/methanol gradient). Part of fraction C (6.2 g) was separated further by HPLC (Prep Pak-500/C₁₈ cartridge; methanol/water 65:35) into eight fractions, C1–C8. Repeated HPLC (Spherisorb 5 and Spherisorb 5 Nitrile) of fraction C3 (272 mg) led to the isola-



Scheme 1. Proposed biogenesis of 1 and 2.

tion of 3.7 mg of (1*S*,2*E*,4*S*,6*R*,7*E*)-4,6-dihydroxy-20-nor-2,7-cembradien-12-one (1). Subsequent separation by HPLC of fraction C4 (343 mg) gave 2.7 mg of (1*S*,2*E*,4*S*,6*R*,7*R*,8*R*)-4,6-dihydroxy-7,8-epoxy-20-nor-2-cembre-12-one (2).

(1*S*,2*E*,4*S*,6*R*,7*E*)-4,6-Dihydroxy-20-nor-2,7-cembradien-12-one (1) was an oil with $[\alpha]_D + 1.5^\circ$ (*c* 0.46, CHCl_3); [Found: ($M-18$)⁺ 290.2216. Calc. for $\text{C}_{19}\text{H}_{30}\text{O}_2$: 290.2245]; IR (CHCl_3): 3602, 3487 and 1709 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 0.83 (d, $J = 6.9$ Hz) / 0.89 (d, $J = 6.7$ Hz) (H-16 / H-17), 1.28 (s, H-18), 1.64 (d, $J = 1.3$ Hz, H-19), 1.79 (dd, $J = 1.5$ and -14.5 Hz, H-5a), 2.21 (dd, $J = 6.4$ and -14.5 Hz, H-5b), 4.70 (m, H-6), 5.42 (dd, $J = 9.2$ and 15.3 Hz, H-2), 5.53 (d, $J = 15.3$ Hz, H-3) and 5.64 (dq, $J = 1.3$ and 9.1 Hz, H-7); MS [m/z (% composition)]: 290 (3, $M-18$), 275 (1, $\text{C}_{18}\text{H}_{27}\text{O}_2$), 247 (7), 229 (3, $\text{C}_{13}\text{H}_{25}\text{O}_3$), 205 (2), 136 (15, $\text{C}_{10}\text{H}_{16}$ and $\text{C}_9\text{H}_{12}\text{O}$), 121 (18, C_9H_{13} and $\text{C}_8\text{H}_9\text{O}$), 109 (19, C_8H_{13} and $\text{C}_7\text{H}_9\text{O}$), 95 (32, C_7H_{11} and $\text{C}_6\text{H}_7\text{O}$), 79 (29, C_6H_7), 69 (22, C_5H_9 and $\text{C}_4\text{H}_5\text{O}$), 55 (40) and 43 (100).

(1*S*,2*E*,4*S*,6*R*,7*R*,8*R*)-4,6-Dihydroxy-7,8-epoxy-20-nor-2-cembre-12-one (2) had m.p. 133–134 $^\circ\text{C}$; $[\alpha]_D - 42^\circ$ (*c* 0.27, CHCl_3); [Found: M^+ 324.2367. Calc. for $\text{C}_{19}\text{H}_{32}\text{O}_4$: 324.2300]; IR (CHCl_3): 3603, 3443 and 1711 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 0.82 (d, $J = 6.8$ Hz) / 0.91 (d, $J = 6.7$ Hz) (H-16 / H-17), 1.28 (s, H-19), 1.32 (s, H-18), 1.99 (dd, $J = 0.9$ and -15.1 Hz, H-5a),

2.23 (dd, $J = 6.7$ and -15.1 Hz, H-5b), 2.91 (d, $J = 9.7$ Hz, H-7), 3.65 (ddd, $J = 0.9, 6.7$ and 9.7 Hz, H-6), 5.36 (dd, $J = 9.6$ and 15.6 Hz, H-2) and 5.66 (d, $J = 15.6$ Hz, H-3); MS [m/z (% composition)]: 324 (0.1, M), 306 (2, $\text{C}_{19}\text{H}_{30}\text{O}_3$), 288 (1, $\text{C}_{19}\text{H}_{28}\text{O}_2$), 273 (1, $\text{C}_{18}\text{H}_{25}\text{O}_2$), 263 (1), 245 (2), 207 (3), 136 (19, $\text{C}_{10}\text{H}_{16}$), 121 (21, C_9H_{13} and $\text{C}_8\text{H}_9\text{O}$), 109 (18), 95 (27, C_7H_{11} and $\text{C}_6\text{H}_7\text{O}$), 81 (22, C_6H_9 and $\text{C}_5\text{H}_5\text{O}$), 69 (23, C_5H_9 and $\text{C}_4\text{H}_5\text{O}$), 55 (35, $\text{C}_3\text{H}_3\text{O}$ and C_4H_7) and 43 (100).

*Treatment of (1*S*,2*E*,4*S*,6*R*,7*E*,8*R*)-4,6-dihydroxy-20-nor-2,7-cembradien-12-one (1) with methyllithium.* To a dry solution of 7.0 mg of 1 in 30 ml of THF/Et₂O (1:1) was added 150 μl of a dry solution of methyllithium in Et₂O (5%). The reaction mixture was stirred under N₂ at -70°C for 30 min. Work-up and chromatography on silica gel (hexane/EtOAc 20:80) gave 0.7 mg of (1*S*,2*E*,4*S*,6*R*,7*E*,12*S*)-2,7-cembradiene-4,6,12-triol (7) and 2.2 mg of the corresponding 12*R*-epimer (5).

Compound 7 was an oil with $[\alpha]_D + 47^\circ$ (*c* 0.07, CHCl_3); IR (CHCl_3): 3603 and 3482 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 0.84 (d, $J = 6.7$ Hz) / 0.90 (d, $J = 6.7$ Hz) (H-16 / H-17), 1.15 (s, H-20), 1.27 (s, H-18), 1.68 (d, $J = 1.4$ Hz, H-19), 1.79 (dd, $J = 2.2$ and -14.4 Hz, H-5a), 2.19 (dd, $J = 6.7$ and -14.4 Hz, H-5b), 4.72 (ddd, $J = 2.2, 6.7$ and 9.1 Hz, H-6), 5.53 (d, $J = 15.3$ Hz, H-3), 5.57 (dd, $J = 8.0$ and 15.3 Hz, H-2) and 5.67

(dq, $J = 1.4$ and 9.1 Hz, H-7); MS (m/z , %): 306 (1, M-18), 288 (3), 273 (1), 263 (1), 245 (4), 227 (2), 217 (2), 205 (2), 189 (2), 177 (4), 161 (6), 151 (5), 136 (31), 121 (20), 109 (24), 94 (53), 79 (33), 69 (30), 55 (30), and 43 (100).

Compound **5** had m.p. 134–135 °C; $[\alpha]_D + 42^\circ$ (c 0.18, CHCl₃); IR (CHCl₃): 3602 and 3460 cm⁻¹; ¹H NMR (CDCl₃): δ 0.86 (d, $J = 6.7$ Hz) / 0.89 (d, $J = 6.6$ Hz) (H-16 / H-17), 1.17 (s, H-20), 1.27 (s, H-18), 1.68 (broad s, H-19), 1.79 (dd, $J = 2.0$ and -14.5 Hz, H-5a), 2.19 (dd, $J = 6.5$ and -14.5 Hz, H-5b), 4.69 (ddd, $J = 2.0, 6.5$ and 8.7 Hz, H-6), 5.51 (d, $J = 15.2$ Hz, H-3), 5.58 (dd, $J = 8.5$ and 15.2 Hz, H-2), and 5.73 (broad d, $J = 8.7$ Hz, H-7); MS (m/z , %): 306 (2, M-18), 288 (7), 273 (2), 245 (7), 227 (3), 217 (3), 205 (2), 189 (3), 177 (6), 161 (9), 151 (6), 136 (43), 121 (29), 109 (33), 94 (100), 79 (60), 69 (39), 55 (36) and 43 (100).

Reduction of (1S,2E,4S,6R,7E,11S,12S)-11,12-epoxy-2,7-cembradiene-4,6-diol (6). A solution of 450 mg of **6**⁶ and an excess of LAH in 50 ml of dioxane was heated under reflux for 0.5 h. Work-up and chromatography on silica gel (hexane/EtOAc 10:90) afforded 400 mg of (1S,2E,4S,6R,7E,12R)-2,7-cembradiene-4,6,12-triol, whose physical and spectral data agreed well with those recorded for **5**.

Reduction of (1S,2E,4S,6R,7E,11R,12R)-11,12-epoxy-2,7-cembradiene-4,6-diol (8). A solution of 24 mg of **8**⁶ and an excess of LAH in 15 ml of dioxane was heated under reflux for 1 h. Work-up and separation by HPLC (Spherisorb 5 Diol, hexane/EtOAc 30:70; Spherisorb 5 Nitrile, hexane/EtOAc 50:50) gave 3.9 mg of (1S,2E,4S,6R,7E,12S)-2,7-cembradiene-4,6,12-triol, whose physical and spectral data were identical to those recorded for **7**.

Epoxidation of (1S,2E,4S,6R,7E)-4,6-dihydroxy-20-nor-2,7-cembradien-12-one (1). To a cold (0 °C) solution of 1.3 mg of **1** in 1 ml of CHCl₃ was added a solution of 2.3 mg of

m-chloroperbenzoic acid in 1 ml of CHCl₃. The solution was kept at 0 °C for 1 h, washed with aqueous NaHCO₃ and water, dried and concentrated. Purification by HPLC (Spherisorb 5, hexane/EtOAc 40:60) gave 0.3 mg of (1S,2E,4S,6R,7R,8R)-4,6-dihydroxy-7,8-epoxy-20-nor-2-cembre-12-one, identical with naturally occurring **2**.

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