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 (1S,2E,4S,6R,7E)-4,6-dihydroxy-20-nor-2,7-cembradien-12-one (1) and (1S,2E,4S,6R,7R,8R)-4,6-dihydroxy-7,8-epoxy-20-nor-2-cembran-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. (1S,2E,4S,6R,7E,11E)-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. We now report the isolation from flowers of Greek tobacco of two 20-norcembranoids (1, 2), the first C19 compounds.

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 spectroscopy 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 sp2 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 (1S,2E,4S,6R,7E,12R)-2,7-cembradiene-4,6,12-triol (5), obtained by LAH-reduction of the 11S,12S-epoxide. The minor product was identified as the corresponding 12S-epimer 7 by comparison with an authentic sample obtained from the 11R,12R-epoxide by reduction using LAH. These results allowed an unequivocal identification of 1 as (1S,2E,4S,6R,7E)-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

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*For Part 66, see Ref. 1.

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results listed in Table 1 and from the $^1$H NMR results listed in Table 2 that the conformation about the 5,6 bond in 1 is reminiscent of that of the 4S,6R,11S-triol but different from that of the 4S,6R-diol. Moreover, the conversion of 1 to the 4S,6R,12R- and 4S,6R,12S-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 $^1$H 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 $\delta$ 1.28 and a one-proton doublet at $\delta$ 2.91. These results, together with the elemental composition, $C_{15}H_{22}O_4$, 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-cembr-12-one. This assignment was readily verified and a 1S,2E,4S,6R-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 (7R,8R)- and (7S,8S)-epoxides 10 and 11. 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 (1S,2E,4S,6R,7R,8R)-4,6-dihydroxy-7,8-epoxy-20-nor-2-cembr-12-one. The $^1$H 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.

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 norterprenoids previously found in
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.

<table>
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<th>Compound</th>
<th>H–5a</th>
<th>H–5b</th>
<th>H–6</th>
<th>H–7</th>
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<tr>
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<td>2.21</td>
<td>4.70</td>
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<td>J_{6,7} = 9.2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.80</td>
<td>2.22</td>
<td>4.67</td>
<td>5.59</td>
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<td>J_{6,7} = 9.1</td>
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<tr>
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<td>2.19</td>
<td>4.69</td>
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<td>J_{5b,6} = 6.8</td>
<td>J_{6,7} = 9.2</td>
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</table>

nature, e.g. 12-norcaryophyllen-2-one (12) and the corresponding epoxide, kubusone (13), of lavender oil. One possible route to 1 and 2, outlined in Scheme 1, would involve the 45,6R,20-triol 14, a recently discovered tobacco constituent, 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 7R,8R-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/C_{18} 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.

Treatment of (1S,2E,4S,6R,7E,8R)-4,6-dihydroxy-20-nor-7,8-epoxy-20-nor-2-cembradien-12-one (I) with methyl lithium. To a dry solution of 7.0 mg of I in 30 ml of THF/Et₂O (1:1) was added 150 μl of a dry solution of methylthyllium 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 (1S,2E,4S,6R,7E,12S)-2,7-cembradiene-4,6,12-triol (7) and 2.2 mg of the corresponding 12R-epimer (8).

Compound 7 was an oil with [α]D + 47° (c 0.07, CHCl₃); IR (CHCl₃): 3603 and 3482 cm⁻¹; 1H NMR (CDCl₃): δ 8.84 (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 (dd, 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), 2.47 (ddd, 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, C₁₉H₂₀O₂), 288 (1, C₁₉H₂₈O₂), 273 (1, C₁₈H₂₆O₂), 263 (1), 245 (2), 207 (3), 136 (19, C₁₇H₁₂), 121 (21, C₉H₁₃ and C₈H₅O), 109 (18), 95 (27, C₇H₁₄ and C₈H₅O), 81 (22, C₆H₀ and C₇H₅O), 69 (23, C₆H₆ and C₇H₅O), 55 (35, C₅H₂O and C₆H₃) and 43 (100).
(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; [α]D + 42° (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° 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° 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-cembrin-12-one, identical with naturally occurring 2.

Acknowledgements. We are grateful to Mr. Jacek Bieławski and Dr. Olof Dahlman who recorded the mass spectra, and to Dr Toshiaki Nishida who recorded the NMR spectra.

References

Received July 3, 1987.