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Tobacco Chemistry. 27. The Structures of Four Stereoisomeric 8,12ξ-Epoxylabd-14-en-13ξ-ols Isolated from Greek *Nicotiana tabacum* L.

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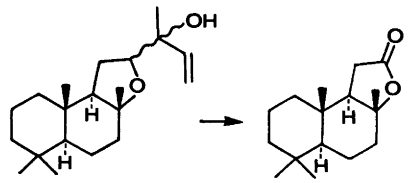
The structures of four stereoisomeric 8,12ξ-epoxylabd-14-en-13ξ-ols** obtained from Greek tobacco were determined by spectroscopic means and by conversion to 12-norambreinolide. Plausible routes for the formation of the new labdanols and related tobacco constituents from a common precursor, abienol, are discussed.

Chemical research on tobacco has revealed the presence of a large number of terpenoids and nor-terpenoids. The vast majority of these either belongs to, or may formally be derived from representatives of three groups of compounds, namely (i) carotenoids – polyisoprenoids, (ii) thunberganes, and (iii) labdanes – drimanes.[1–5] This paper deals with the structural elucidation of four new representatives of the labdane group.

GC-MS examination of a medium-polar, neutral fraction ('B 5') of an extract of Greek tobacco [6] revealed the presence of four compounds which exhibited nearly identical mass spectra suggesting that they were stereoisomers. Two of the isomers (*1a* and *1b*) occurred in quantities permitting isolation and subsequent structure determination. Accurate mass determinations of the M – 15 and M – 18 ions, and the appearance of twenty ^{13}C-resonances in the CMR spectrum of stereoisomer *1b*, established the elemental composition as $C_{20}H_{34}O_2$. Furthermore, the mass spectrum was indicative of a labdane diterpenoid-structure devoid of additional substituents in ring A, [7] *e.g.* ions at m/e 137, 177, and 191. The NMR-spectrum of the stereoisomer *1b* displayed five three-proton singlets in the δ 0.8 – 1.15 region corresponding to three methyl groups on quaternary carbon atoms, and two on oxygenated, tertiary carbons. An ABC-signal pattern in the δ 5.0 – 5.9 region characteristic of a vinyl group bound to a fully substituted carbon was also observed as was a one-proton quartet at δ ~3.80 (*J ca.* 5 and 7 Hz), attributed to a proton on an oxygenated carbon atom. These data made two structures appear likely, namely 8,12ξ-epoxylabd-14-en-13ξ-ol (*1*) and 8,13ξ-epoxylabd-14-en-12ξ-ol (*3*, R = OH). The former of these was favoured since all four stereoisomeric tobacco compounds exhibited a medium intense *m/e* 235 ion ($C_{16}H_{27}O$), only readily rationalized in terms of structure *1* [favoured C(12) – C(13) cleavage], and gave mass spectra differing from that of 8,13β-epoxylabd-14-en-12α-ol. [8]



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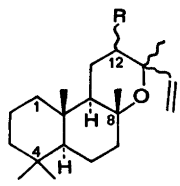

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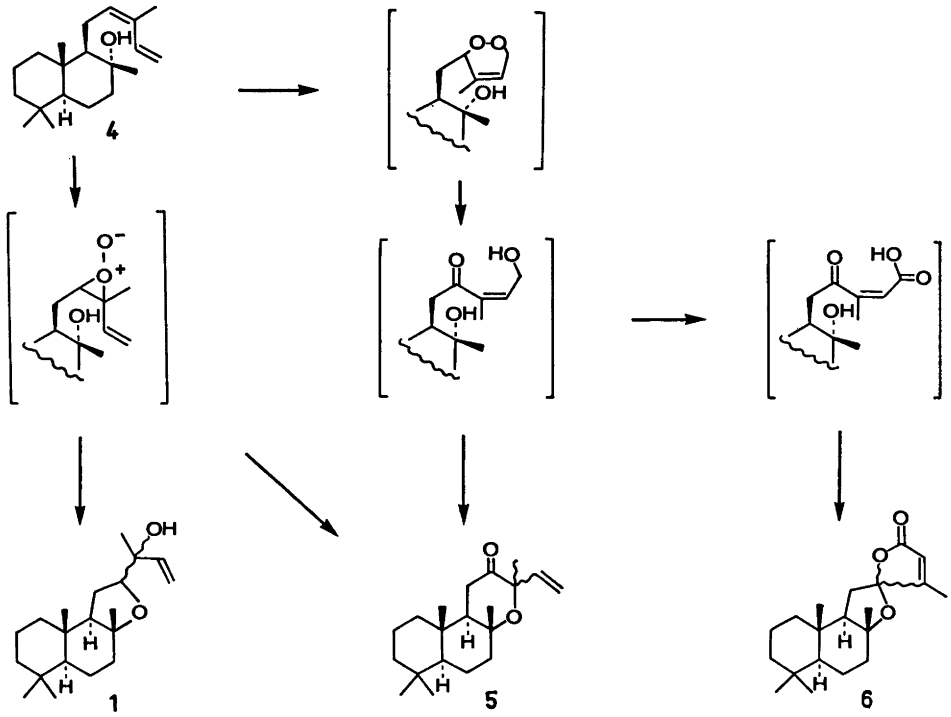
** Nomenclature according to J. W. Rowe, Oct. 1968; personal communication.

The spectral results were verified chemically since neither of the two main tobacco compounds (*1a*, *1b*) could be acetylated under mild conditions (Ac_2O/py) and oxidation (employing various methods involving CrO_3) did not give ketones, but furnished 12-norambreinolide [9] (*2*) in almost quantitative yield. The latter transformation confirmed the larger part of the carbon skeleton and established the absolute configurations at C(5), C(8), C(9) and C(10); the validity of the 8*R* assignment follows from the facts that the C(8) epimer of the lactone (*2*) has been shown to be the thermodynamically more stable isomer [10] and that authentic 12-norambreinolide (*2*) was virtually unaffected when treated under the same conditions as employed for the oxidation. The above spectral data show how the five carbons beyond C(11) in the side chain are linked and also that C(12) is secondary and carries an ether oxygen function and that C(13) is tertiary and carries a methyl, a vinyl and an oxygen substituent. The only additional structure, 12ξ,13ξ-epoxylabd-14-en-8-ol, consistent with these results, could be excluded on the basis that the proton at C(12) in this compound should resonate at notably higher field (δ 2.5 – 3.2) [11] than actually observed, *i.e.* δ 3.8. Furthermore, the structures of the major stereoisomer (*1b*), and one of the minor ones (*1d*), have recently been confirmed in our laboratory by synthesis, which will be discussed elsewhere.[12] The structure of the fourth isomer (*1c*), however, rests presently on mass spectral evidence only.

The new diterpenoids have the same absolute configuration at C(5), C(8), C(9), and C(10) as the other labdanes previously isolated from tobacco, and are, as the majority of these, oxygenated at C(8) and C(12). The co-occurrence of several sets of labdanoid diastereomers differing only with respect to the side-chain stereochemistry suggests that they are formed from a labdane precursor such as abienol (*4*), recently isolated from tobacco, [13] by non-stereospecific oxidation. Plausible routes for the formation of the new compounds (*1a* – *1d*), and some earlier encountered representatives – the α- and β-levantenolides (*6*) [14] and two 13-



Scheme 1.

epimeric 8,13-epoxylabd-14-en-12-ones (*5*) [15] – involving commonly accepted singlet oxygen intermediates, [16,17] are depicted in Scheme 1. Earlier published views on the biosynthesis of the α- and β-levantenolides differ from ours in assuming that oxidation occurs prior to cyclizations and that the actual precursor is the appropriate acyclic keto-acid. [18,19] It is of interest to note that the new compounds (*1a–1d*) in turn constitute potential precursors for 12-norambreinolide (*2*), which was first identified as a constituent of the aroma of cigar tobacco,[20] and also a series of nor- and seco-terpenoids very recently obtained from Greek tobacco.[5,21]

EXPERIMENTAL

NMR, IR, and mass spectra were recorded on Varian HA 100D and XL 100, Digilab FTS-14 and Perkin-Elmer 257, and LKB 9000 (70 eV) instruments, respectively. Rotations were measured on a Perkin-Elmer 141 instrument. Accurate mass determinations were carried out at the laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm. The mass spectra were obtained by gas chromatography in combination with the mass spectrometer (GC-MS). Analytical gas chromatography was performed on a Varian 1700 instrument using either a steel or a glass capillary column coated with emulphor and Ucon Oil HB 2000, respectively.

Isolation. Fraction B5 [6] was subjected to distillation using CO_2 as carrier gas, furnishing a less volatile fraction which was further subdivided employing column chromatography on silica gel and $AgNO_3$-impregnated silica gel. Thus stereoisomers *1a* (*ca.* 30 mg; numbering *1a* to *1d* according to increasing retention times, glass capillary column coated with HB 2000) and *1b* (*ca.* 200 mg) were obtained in the pure state, while the quantities of stereoisomers *1c* and *1d* only permitted detection by GC-MS. The subfractionation of B5 will be reported elsewhere.

Stereoisomer 1a. δ(CCl_4): 0.81 (3 H, s), 0.82 (3 H, s), 0.87 (3 H, s), 1.10 (3 H, s), 1.18 (3 H, s), 3.80 (1 H, dd, *J* 5 and 7.5 Hz), 5.02 (1 H, dd, *J* 2 and 10 Hz), 5.22 (1 H, dd, *J* 2 and 17.5 Hz), 5.75 (1 H, dd, *J* 10 and 17.5 Hz); ν(film): 3470 (broad), 2974 (s), 2932 (s), 2870 (s), 1461 (m), 1380 (m), 1369 (m), 1152 (m), 1122(s), 1080 (m), 1004 (m), 985 (m), 930 (m), 870 (w), 845 (w); MS: *m/e* 306 (M+, not observed), 191 (100), 69 (38), 137 (37), 109 (32), 95 (26), 81 (26), 41 (24), 43 (23), 55 (22), 192 (19), 217 (18), 235 (17); accurate mass determination: $C_{19}H_{31}O_2$ (M – 15), found 291.2316, calc. 291.2324; $[\alpha]^{20}$ (*c* 0.57; $CHCl_3$), 436 nm: +32.5°, 546 nm: +14.4°, 578 nm: +130°, 589 nm: +12.3°.

Stereoisomer 1b. δ(CCl_4): 0.82 (6 H, s), 0.87 (3 H, s), 1.10 (3 H, s), 1.15 (3 H, s), 2.26 (OH), 3.82 (1 H, dd, *J* 5.5 and 6.5 Hz), 5.00 (1 H, dd, *J* 2 and 10.5 Hz), 5.21 (1 H, dd, *J* 2 and 17.5 Hz), 5.86 (1 H, dd, *J* 10.5 and 17.5 Hz); δ (^{13}C, ppm from internal TMS, in C_6H_6:$CDCl_3$ 1:1, tentative assignments are based on those of manoyl oxide [22]) 39.6 (C-1), 18.4 (C-2), 42.3 (C-3),32.9 (C-4), 57.1 (C-5), 20.1 (C-6), 39.7 (C-7), 73.8 (C-13), 59.7 (C-9), 36.4 (C-10), 23.9 (C-11), 81.1 (C-12), 80.5 (C-8), 142.1 (C-14), 111.5 (C-15), 21.2 (C-17), 23.5 (C-16), 33.3 (C-18), 21.1 (C-19), 14.9 (C-20); ν(film): 3470 (broad), 2929 (s), 2870 (m), 2849 (m), 1461 (m), 1378 (m), 1368 (m), 1325 (w), 1300 (w), 1275 (w), 1255 (w), 1208 (w), 1198 (w), 1181 (w), 1162 (w), 1125 (m), 1080 (m), 1033 (w), 1005 (s), 987 (m), 925 (m), 880 (w), 869 (w), 845 (w), 828 (w); MS: *m/e* 306 (M+, not observed), 191 (100), 69 (38), 137 (36), 109 (31), 81 (26), 95 (25), 41 (23), 43 (22), 55 (22), 192 (18), 217 (17), 235 (15); accurate mass determinations: $C_{19}H_{31}O_2$ (M – 15), found 291.2315, calc. 291.2324; $C_{20}H_{32}O$, found 288.2447, calc. 288.2453; $C_{16}H_{27}O$, found 235.2070, calc. 235.2062; $C_{16}H_{25}$, found 217.1963, calc. 217.1956; $C_{14}H_{23}$, found 191.1802, calc. 191.1800; $[\alpha]^{20}$ (*c* 0.45; $CHCl_3$), 365 nm: −0.2°, 436 nm: −0.3°, 546 nm: −0.3°, 578 nm: −0.3°, 589 nm: −0.2°.

Stereoisomer 1c. MS: *m/e* 306 (M+, not observed), 191 (100), 69 (39), 137 (37), 109 (32), 55 (30), 95 (27), 81 (26), 41 (26), 43 (25), 192 (24), 217 (23), 123 (20), 235 (19).

Stereoisomer 1d. MS: *m/e* 306 (M+, not observed), 191 (100), 69 (54), 43 (50), 137 (47), 41 (45), 109 (43), 95 (37), 81 (36), 55 (33), 192 (24), 217 (22), 235 (21).

Acetylation. Stereoisomer *1b* (5 mg) in dry pyridine (1 ml) was added to a solution of acetic anhydride (0.5 ml) in dry pyridine (1 ml) and the mixture kept at room temperature for 24 h. Excess anhydride was destroyed by the addition of a few drops of methanol. Water was added and the mixture extracted with ether and the extract washed with diluted H_2SO_4, $NaHCO_3$ and water. Concentration of the extract left a colourless oil (4 mg) indistinguishable (TLC) from the starting material.

Oxidation. Treatment of stereoisomers *1a* and *1b*, respectively, with Jones' ($CrO_3 - H_2SO_4 - H_2O$ – acetone),[23] Collins' (dipyridine – CrO_3 (*IV*) – CH_2Cl_2),[24] a two-phase (ether – CrO_3 – H_2O) system,[25] or CrO_3 – 80 % acetic acid furnished a single product which was only slightly less polar (TLC, SiO_2, 30 % ether-pentane) than the starting material (R_F = 0.30 and 0.24, respectively). For instance, stereoisomer *1b* (13 mg) when stirred with CrO_3 (30 mg) in 80 % acetic acid (1 ml) at ambient temperature for 2 h afforded after dilution with aqueous $NaHCO_3$, extraction with ether and distillation of the solvent, a colourless oil (10 mg) which crystallized on standing and was found indistinguishable from authentic 12-

norambreinolide (*2*); m.p. 124 °C (lit.[20] 125–125.5 °C) the IR [10] and mass spectra [26] were found identical to those of authentic 12-norambreinolide (*2*); $[\alpha]^{20}$, *2* prepared from stereoisomer *1a* (*c* 0.1; $CHCl_3$), 365 nm: +166°, 436 nm: +92° 546 nm: +48°, 578 nm: +44° 589 nm: +36°; *2* prepared from stereoisomer *1b* (*c* 0.5; $CHCl_3$), 365 nm: +185°, 436 nm: +107°, 546 nm: +58°, 578 nm: +50°, 589 nm: +48°; authentic 12-norambreinolide (*c* 0.46; $CHCl_3$), 365 nm: +180°, 436 nm: +104°, 546 nm: +57°, 578 nm: +49°, 589 nm: +47°.

Stability-test of 12-norambreinolide (2). The lactone (*2*, 5 mg) in acetic acid (1 ml) was stirred at room temperature for 2 h in the presence of CrO_3 (30 mg). The mixture was worked up as outlined (*vide supra*) and the products (2 mg) examined by GC-MS which revealed the presence of 12-norambreinolide and 8-epi-12-norambreinolide in the ratio 99:1.

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