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Tobacco Chemistry

13, 8,13-Epoxyabd-14-en-12-one and 8,13 β -Epoxyabd-14-en-12-one,* Two New Diterpenoids from Tobacco

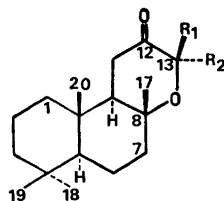
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Two new diterpenoids have been isolated^{1,2} from sun-cured Greek tobacco, *N. tabacum*, and here assigned structures I and II for reasons given below.

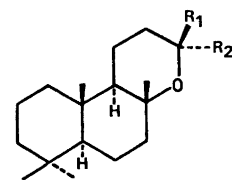
The mass spectra of the two compounds were closely similar, indicating that they were diastereoisomers, and accurate mass determinations established the elemental

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



I R₁: CH₃; R₂: CH=CH₂

II R₁: CH=CH₂; R₂: CH₃



III R₁: CH₃; R₂: CH₂CH₃

IV R₁: CH₂CH₃; R₂: CH₃

V R₁: CH₃; R₂: CH=CH₂

VI R₁: CH=CH₂; R₂: CH₃

compositions of both as C₂₀H₃₂O₂. The fragmentation patterns were strongly reminiscent of those of manoyl oxide³ (8,13-epoxyabd-14-ene, V) and 13-epimanoyl oxide³ (8,13 β -epoxyabd-14-ene, VI), suggesting that they were 12-oxo derivatives of these or of closely related compounds. Thus, the ions *m/e* 234, 206, 192, and 177 could be attributed to the ions A, B, C, and C-15 as depicted in Fig. 1 on the basis of the previously reported³ fragmentation patterns of the deoxo compounds. The elemental compositions of these ions were determined by high-resolution mass measurement. The presence of a non-conjugated six-membered ring ketone, a vinyl group, and five methyl groups were evident from UV, IR, and NMR⁴ spectra supporting the proposed structures.

The oxo groups of the new compounds were therefore removed by the Huang-Minlon procedure.⁵ In both cases, this also resulted in partial reduction of the vinyl group analogous to the results previously obtained by Giles *et al.*⁶ Due to shortages of material, both mixtures were hydrogenated to give homogeneous products. The two deoxodihydro derivatives exhibited optical rotations, IR and NMR spectra which were identical to those of 8,13-epoxyabdane

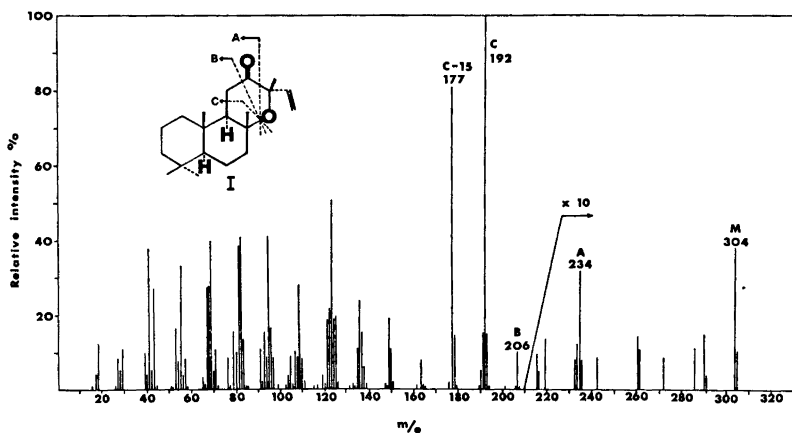


Fig. 1. Mass spectrum of 8,13-epoxylabd-14-en-12-one.

(III) and 8,13 β -epoxylabdane (IV), respectively, thereby proving the carbon skeleton and the stereochemistry of the new compounds since the absolute configurations of manoyl oxide (V) and 13-epimanoyl oxide (VI) have been elucidated.⁷

With the structures and stereochemistry of the deoxo derivatives established, the location of the carbonyl group follows from the mass-spectral data mentioned and from evidence acquired from the NMR spectra. These spectra suggested the presence of one methylene group (δ ca. 2.2–2.5) adjacent to the carbonyl group. The chemical shifts and coupling constants of this group were examined directly in the case of II and after the addition of a shift reagent⁸ ('Eu(fod)₃d₂₇') in the case of I. The methylene protons were shown to constitute the AB part of an ABC system (see Experimental) demonstrating the presence of a $>CH-CH_2-CO-$ moiety. Such a grouping is possible only with the carbonyl group in position 7 or 12. The former alternative, also ruled out for mass-spectral reasons, could be excluded on account of the large lanthanide-induced shift of the vinyl protons. Thus it follows that the two tobacco constituents are 8,13-epoxylabd-14-en-12-one (I) and 8,13 β -epoxylabd-14-en-12-one (II).

Successive additions of shift reagent⁸ to the NMR solution of I allowed the following assignments of the methyl groups: δ 0.78, C(20)H₃; δ 0.81, C(18)H₃; δ 0.90, C(19)H₃; δ 1.15, C(17)H₃; δ 1.34, C(16)H₃. Since II displays almost identical chemical shifts

these assignments are probably also valid for this compound.

8,13 β -Epoxylabd-14-en-12 α -ol has previously been isolated from Turkish tobacco leaves⁶ and smoke.⁹ The physical properties (m.p., IR, NMR) of the corresponding ketone obtained on oxidation^{6,10} agreed well with those of II. The presence of the two epimers I and II was not unexpected in view of the previously observed co-occurrence of the related pairs manoyl oxide/13-epimanoyl oxide and 8 β ,13-epoxylabd-14-ene/8 β ,13 β -epoxylabd-14-ene in other plants.^{11–13}

Experimental. Melting points (uncorrected), NMR, IR, UV, and mass spectra were recorded on Leitz Wetzlar, Varian HA-100D, Digilab FTS-14, Beckmann DK-2A, and LKB 9000 (70 eV) instruments, respectively. Accurate mass determinations were carried out at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm.

8,13-Epoxylabd-14-en-12-one (I). The isolation of I (6.3 mg) is described elsewhere.¹ M.p. 82–82.5°; $[\alpha]_D^{20}$ –142° (c 0.26 in chloroform); IR (film): 1708 (s), 1633 (m), 1390 (m), 1381 (m), 1221 (m), 1180 (m), 1156 (m), 1120 (m), 1100 (m), 1084 (w), 1030 (m), 989 (w), 924 (m), 895 (w) cm⁻¹; MS: see Fig. 1; accurate mass determination: C₂₀H₃₂O₂, found 304.2393, calc. 304.2402; NMR (CDCl₃, TMS internally): δ 0.78 (3H,s), 0.81 (3H,s), 0.90 (3H,s), 1.15 (3H,s), 1.34 (3H,s), ca. 2.03 (H, J =7.3 and 12.0 Hz, ca. 2.22 (H, J =12.0 and –18.0 Hz), ca. 2.48 (H, J =7.3 and –18.0 Hz), 5.15 (H, J =1.5 and 10.5 Hz), 5.47 (H, J =1.5 and 16.6 Hz), 5.74

(H, $J=10.5$ and 16.6 Hz). The coupling constants of the protons resonating at δ ca. 2.22 and 2.48 were measured after addition of 'Eu(fod)₃d₂₇'.

8,13 β -Epoxyabd-14-en-12-one (II). The isolation of II (11.8 mg) is described elsewhere.¹ M. p. 93–93.5°, lit.⁶ 92–93°; $[\alpha]_D^{20} + 90^\circ$ (c 0.39 in chloroform); IR (KBr): 1707 (s), 1640 (w), 1262 (w), 1228 (w), 1222 (w), 1205 (m), 1132 (s), 1086 (s), 1045 (m), 1028 (m), 1001 (s), 990 (s), 976 (m), 922 (s), 906 (w), 864 (w), 846 (w) cm^{-1} , the IR bands corresponded well with those communicated by Dr. Giles;¹⁰ UV (ethanol): no absorption above 200 nm; mass spectrum, m/e (%): 304 (3), 289 (2), 286 (1), 261 (7), 234 (3), 206 (10), 192 (100), 177 (82), 137 (23), 136 (23), 123 (51), 109 (27), 95 (41), 82 (40), 81 (36), 69 (38), 63 (28), 67 (27), 55 (31), 41 (34), these data accorded well with those provided by Dr. Giles;¹⁰ accurate mass determinations: C₂₀H₃₂O₂, found 304.2391, calc. 304.2402; C₁₆H₂₀O, found 234.1982, calc. 234.1984; C₁₅H₂₆, found 206.2025, calc. 206.2034; C₁₄H₂₄, found 192.1890, calc. 192.1878; C₁₃H₂₁, found 177.1637, calc. 177.1643; NMR (CDCl₃, TMS internally): δ 0.79 (3H,s), 0.82 (3H,s), 0.90 (3H,s), 1.18 (3H,s), 1.31 (3H,s), ca. 1.90 (H, $J=6.5$ and 12.0 Hz), 2.33 (H, $J=12.0$ and -18.0 Hz), 2.47 (H, $J=6.5$ and -18.0 Hz), 5.04 (H, $J=1.3$ and 10.3 Hz), 5.16 (H, $J=1.3$ and 17.0 Hz), 6.12 (H, $J=10.3$ and 17.0 Hz).

Wolff-Kishner reduction of 8,13-epoxyabd-14-en-12-one (I). I (2.6 mg), diethylene glycol (1 ml), and hydrazine hydrate (99.5 %, 0.1 ml) were heated to 110° for 1 h in a small test tube fitted with an air condenser. Potassium hydroxide (85 mg) was added and the mixture kept at 195–200° for 5 h. The solution was cooled, diluted with water, and extracted with ether. The excess hydrazine hydrate and water which was collected from the condenser was also extracted with ether since some of the product had apparently steam distilled. The combined extracts (3.5 mg), which consisted (TLC) of a mixture of manoyl oxide (V) and 8,13-epoxyabdane (III), were concentrated, dissolved in ethanol (2 ml), and hydrogenated for 2 h at ambient temperature using 10 % Pd/C (20 mg) as catalyst. The product was extracted with ether and chromatographed on a small silica gel column to give 8,13-epoxyabdane (III, 1.5 mg): $[\alpha]_D^{20} + 6.7^\circ$ (c 0.15 in chloroform); $[\alpha]_D^{20} + 5.9^\circ$ (c 1.1 in chloroform) for 8,13-epoxyabdane prepared here by hydrogenation of authentic manoyl oxide; MS, IR (film) and NMR (CDCl₃) were identical to those of 8,13-epoxyabdane,³ the methyl signals of the latter spectrum were found at δ 0.78 (s), 0.80 (s), 0.85 (t), 0.86 (s), 1.18 (s) and 1.28 (s).

Wolff-Kishner reduction of 8,13-epoxyabd-

14-en-12-one (II). II (3.9 mg) was reduced as described above using the same amounts of reagents. The mass spectrum of the product (2.7 mg), obtained after purification on a small silica gel column, indicated the presence of a mixture of both 13-epimanoyl oxide (VI) and 8,13 β -epoxyabdane (IV). Thus the mixture was hydrogenated as outlined above giving an apparently homogeneous (TLC) product (2.3 mg): $[\alpha]_D^{20} + 23^\circ$ (c 0.11 in chloroform), lit.^{6,14} $[\alpha]_D^{20} + 21.1^\circ$ and 17° ; m.p. 22°, lit.⁶ 26–28°; MS, IR (film) and NMR (CDCl₃) were identical to those of authentic 8,13 β -epoxyabdane (IV) prepared here by hydrogenation of 13-epimanoyl oxide (VI), the methyl signals of the latter spectrum were found at δ 0.78 (s), 0.80 (s), 0.86 (s), 0.88 (t), 1.08 (s) and 1.26 (s).

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