

Tobacco Chemistry

21. Three New Volatile Tobacco Constituents of Probable Isoprenoid Origin

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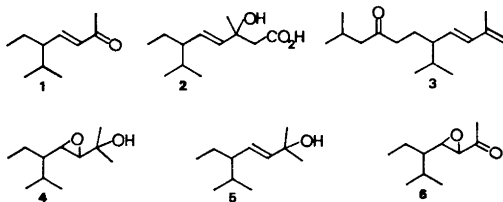
We recently reported the isolation of the three new naturally occurring compounds 1, 2, and 3 from the volatile fraction of an extract from Greek tobacco, *Nicotiana tabacum* L.¹ Our continued interest in tobacco flavour constituents of terpenoid origin has led to the identification of three new compounds structurally related to 1 and 2. The isolation, structural elucidation, and synthesis of these constitute the subject of the present communication.

The first two compounds were diastereomers isolated as a mixture which on high resolution gas chromatography in combination with mass spectrometry (GC-MS) was partially resolved into two compounds exhibiting almost identical mass spectra. The elemental composition of the heaviest fragment observed in the mass spectrum, m/e 171 ($M-15$), indicated the formula $C_{11}H_{22}O_2$ for the two diastereomers. The presence of a tertiary hydroxyl group was apparent from IR absorption at 3450 cm^{-1} , two methyl singlets at δ 1.38 and δ 1.43, and the absence of signals in the δ 3.0–4.5 region in the NMR spectrum. On addition of $\text{Eu}(\text{fod})_3$, these two magnetically non-equivalent methyl groups gave rise to four distinct, strongly downfield-shifted methyl signals providing further evidence for the diastereomeric nature of the mixture.

A *trans* oxirane ring accounted for the second oxygen atom as judged from resonances in the NMR spectrum appearing as two sets of signals due to the presence of two diastereomers: doublet of doublets at δ 2.87 and δ 2.88 (J 2.5 and 8.5 Hz), and doublets at δ 2.73 and δ 2.74 (J 2.5 Hz). The J_{vic} , 2.5 Hz for the protons on both epoxides is consistent with *trans* disubstitution since spin-spin coupling constants across oxirane rings possessing no electronegative substituents have invari-

ably been found in the range +1.9 to +2.5 Hz and +4.0 and +5.0 Hz for *trans* and *cis* configurations,^{2,3} respectively. The presence of the electronegative hydroxyl function one carbon atom removed from the coupling path would be expected to cause an increase in J_{vic} for the oxirane protons,⁴ further supporting the *trans*-disubstituted epoxide assignment on the basis of the observed J_{vic} . The trisubstituted carbon atom α to the oxirane ring carried either a methyl and a *sec*-butyl group, or an ethyl and an isopropyl group which were indicated in the NMR spectrum as two overlapping doublets at δ 0.97 and δ 0.99 (J 7 Hz), and an obscured triplet at δ 0.99 (J ca. 7 Hz). Since the induced shift ratios⁵ observed on addition of the shift reagent were of the same magnitude for these signals the latter alternative, structure 4 was favoured for these new tobacco compounds. Strong m/e 59 and 85 ions in the mass spectrum arising from cleavages α to the epoxide group support the proposed structure. The dominant m/e 72 ion could be ascribed to transannular cleavage of the oxirane ring.⁶

Synthesis of the racemic epoxyalcohol 4 confirmed the proposed gross structure (4) and stereochemical assignments. Treatment of the (\pm)-ketone 1¹ with methylmagnesium iodide in ether solution gave the alcohol 5, which on overnight reaction with *m*-chloroperbenzoic acid in methylene chloride gave 4 as a mixture of diastereo-



mers. The synthetic mixture appeared as two partially resolved peaks on high resolution gas chromatography and did not separate from the natural products on co-injection. The NMR, IR, and mass spectra of the synthetic and natural materials were indistinguishable.

The occurrence of the epoxyalcohols 4 in the tobacco extract prompted us to investigate if the related compounds 5 and 6 were also present in Greek tobacco. A search of our mass spectral data located a

minor component in fraction B-3,^{7,8} having a mass spectrum identical to that of authentic (\pm)-6, conveniently prepared by epoxidation of the ketone *I* with 30 % hydrogen peroxide in alkaline methanolic solution. The natural product was isolated by preparative gas chromatography and its structure confirmed by comparison of its NMR, IR, and mass spectra with those of the synthetic epoxyketone *6*, and co-injection on a capillary GC-column with authentic *6*. It was not clear from NMR or high resolution gas chromatography whether the natural epoxyketone *6* was a diastereomeric mixture. We have not as yet been able to conclusively identify the alcohol *5* as a component of Greek tobacco.

Experimental. Materials and methods. NMR, IR, and mass spectra were recorded on Varian HA 100D and A60-A, Digilab FTS-14, and LKB 9000 (70 eV) instruments, respectively. Columns used for combined capillary GC-MS, analytical and preparative GC, and purification of solvents, silica gel, and drying agents were as previously described.¹

Isolation. The extraction and fractionation of the Greek tobacco (*N. tabacum* L.) has previously been described.⁷ Compounds *4* and *6* were isolated by column chromatography on silica and silica impregnated with 20 % AgNO₃, followed by preparative GC, from fractions B-5,⁷ and B-3,^{7,8} respectively.

3,4E-Epoxy-5 ξ -isopropyl-2-methylheptan-2-ol (4, 10 mg). MS: M⁺ at *m/e* 186 not observed, 43 (100), 72 (66), 85 (49), 59 (43), 41 (42), 55 (40), 69 (36), 57 (35), 83 (15); accurate mass measurement: C₁₀H₁₈O₂ (M-15), found 171.1383, calc. 171.1385; δ (CDCl₃): 0.97 (3H, d, *J* 7 Hz), 0.99 (3H, d, *J* 7 Hz), 0.99 (3H, t, *J* 7 Hz), 1.38 (3H, s), 1.43 (3H, s), 1.1–2.0 (4H, m), 2.74 (1H, d, *J* 2.5 Hz), 2.88 (1H, dd, *J* 2.5 and 8.5 Hz); oxirane protons of second diastereomer: δ 2.73 (1H, d, *J* 2.5 Hz), 2.87 (1H, dd, *J* 2.5 and 8.5 Hz). Addition of Eu(fod)₃: *r* (relative induced shift ratio⁵) = 1 and 1.13 (C(2) (CH₃)₂) for the first diastereomer, and *r* = 1.04 and 1.21 for the second isomer. ν (film): 3450 (broad), 1255 (m), 1150 (broad), 965 (m), 910 (s), 815 (m) cm⁻¹.

3,4E-Epoxy-5 ξ -isopropylheptan-2-one (6, 1 mg). MS: 170 (M⁺, 0.2), 85 (100), 43 (83), 41 (33), 55 (30), 57 (30), 69 (17); accurate mass measurement: C₁₀H₁₈O₂ (M-15), found 155.1065, calc. 155.1072; δ (CDCl₃) 0.97 (6H, 2d, *J* 6.5 Hz), 0.96 (3H, obscured), 1.1–2.0 (4H, m), 2.04 (3H, s), 2.9 (1H, dd, *J* 2.0 and 8.0 Hz), 3.16 (1H, d, *J* 2.0 Hz); ν (film): 1715 (s), 1360 (m), 1250 (m), 875 (m) cm⁻¹.

Synthetic products. (\pm)-5-Isopropyl-2-methylhept-3E-en-2-ol (*5*). (\pm)-5-Isopropylhept-3E-en-2-one¹ (*I*), 1.54 g) in dry ether (5 ml) was added to a suspension of methylmagnesium iodide (from 0.26 g Mg, 10 % excess) in ether (20 ml) and the reaction mixture refluxed for 30 min. Decomposition of the complexes with cold dilute H₂SO₄ and ether extraction gave essentially pure *5* (1.6 g). Material purified by column chromatography on silica gel gave MS: 170 (M⁺, 0.1), 43 (100), 71 (45), 85 (19), 41 (18), 59 (14), 55 (13), 69 (11), 39 (7), 101 (7); ν (film): 3380 (broad), 1150 (m), 975 (s) cm⁻¹.

(\pm)-3,4E-Epoxy-5-isopropyl-2-methylheptan-2-ol (*4*). *m*-Chloroperbenzoic acid (330 mg) was added to a solution of the allylic alcohol (*5*, 300 mg) in methylene chloride (10 ml). After 3 h at room temperature TLC showed no unreacted starting material and the reaction mixture was worked up to yield, after column chromatography on silica, TLC-pure (\pm)-epoxy-alcohol (*4*, 210 mg). The synthetic mixture of diastereomers (ratio 2:3) was shown to be identical to the mixture of diastereomers of *4* isolated from the tobacco by their identical NMR, IR, and mass spectra and successful co-injection on a capillary GC column.

(\pm)-3,4E-Epoxy-5-isopropylheptan-2-one (*6*). Aqueous 1 M NaOH solution (2 ml) was added over 4 h to a stirred solution of (\pm)-5-isopropylhept-3E-en-2-one (*I*, 300 mg) and 30 % hydrogen peroxide (0.6 ml) in methanol (4 ml) at room temperature. After 5 h total reaction time TLC indicated no unreacted starting material and the reaction mixture was worked up to yield, after chromatography on silica, pure (\pm)-*6* (250 mg) as a colourless liquid. The NMR, IR, and mass spectra of synthetic (\pm)-*6* were indistinguishable from those of *6* isolated from the tobacco and the compounds did not separate when co-injected on a capillary GC-column.

(\pm)-3-Isopropylpent-1E-en-1-yl acetate (*7*). From the overnight reaction of (\pm)-5-isopropylhept-3E-en-2-one (*I*) with *m*-chloroperbenzoic acid in methylene chloride solution at room temperature in the absence of added alkali the vinyl acetate *7* was isolated in 30 % yield after column chromatography on silica. δ (neat): 0.80 (3H, d, *J* 6.5 Hz), 0.86 (3H, d, *J* 6.5 Hz), ca. 0.83 (3H, obscured) 1.0–1.9 (4H, m), 2.02 (3H, s), 5.06 (1H, dd, *J* 9 and 12.5 Hz), 6.99 (1H, d, *J* 12.5 Hz); ν (film): 1760 (s), 1674 (m), 1225 (s), 1090 (s), 940 (m) cm⁻¹.

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On the Structures of Two Crystalline Forms of Oxydiacetic Acid

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The structure of the oxydiacetate group has been investigated in several compounds. It has been determined as hydrogen oxydiacetate ion in alkali salts,^{1,2} and as oxydiacetate ion in cadmium³ and lanthanoid⁴ compounds. In these structures the ligand is planar, except in one of the three known cadmium oxydiacetate phases.³ In the alkali compounds, hydrogen bond systems with very short hydrogen bonds were found.^{1,2} We considered it therefore to be of interest to examine the free acid especially with regard to planarity and hydrogen bonds.

From aqueous solutions of oxydiacetic acid two different crystalline phases, one orthorhombic and one monoclinic, are formed side by side at room temperature. In order to determine space groups and preliminary cell dimensions, rotation and Weissenberg photographs were taken for both phases. Some important crystal data are summarized in Table 1. The orthorhombic crystals are efflorescent at room temperature. From the observed density, 1.54 g cm⁻³, and the cell volume given in Table 1, it is concluded that there is at least one water of crystallization in this compound.

Powder photographs for the monoclinic phase were obtained from a Guinier-Hägg camera with CuK α_1 radiation and aluminium (cubic $a=4.04934$ Å) as internal standard. Least-squares refinement of the data gave the accurate cell constants shown in Table 1.

Table 1. Crystal data for the two phases of oxydiacetic acid.

Space group	Monoclinic <i>C2/c</i>	Orthorhombic <i>Pna2₁</i> or <i>Pnam</i>
<i>a</i>	9.706(2) Å	7.52 Å
<i>b</i>	3.941(1)	8.24
<i>c</i>	15.027(2)	10.84
β	104.79(2)°	—
<i>V</i>	555.75 Å ³	671 Å ³
<i>Z</i>	4	4

Powder photographs of the orthorhombic phase, showed, among other lines, also those characteristic of the monoclinic phase. The orthorhombic crystals are thus rather quickly transformed into the monoclinic phase, when powdered. For this reason we have not been able to refine the orthorhombic cell constants.

So far we have determined the structure of the monoclinic phase only. Three-dimensional intensity data have been collected by the use of the Weissenberg multiple film technique with CuK α radiation. The number of observed, independent reflexions was 396. The method of symbolic addition was used (GAASA) for the structure determination. The systematic extinctions were consistent with the space groups *C2/c* (No. 15) and *Cc* (No. 9). The *E* statistics were decidedly in favour of the centrosymmetrical space group *C2/c*.

The positions of the oxygen and carbon atoms were obtained from the *E* map. It