Synthesis and Enantiodifferentiation of Isomeric 2,3,5,6,8,8a-Hexahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-ones (3,4-Dihydro-3-oxoedulans)

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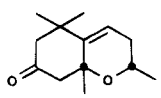
The benzopyran derivatives **1a–d** were prepared in a biomimetic type reaction from their presumed natural precursor 3-oxo-*retro*-α-ionol, which was available from α-ionone by *tert*-butyl chromate oxidation, reduction with $NaBH_4$, and subsequent rearrangement of the 7,8 double bond. The so-obtained geometrical isomers of 3-oxo-*retro*-α-ionol (**4a/b**) were separated by preparative and analytical multilayer coil countercurrent chromatography. Racemic 3-oxo-*retro*-α-ionol (**4a**) was esterified with (*R*)-(−)-2-phenylpropionic acid, and the resulting diastereomeric esters (**5a/b**) were isolated in pure form by preparative HPLC. Configuration at C-9 was determined by ^1H NMR spectroscopy. The isomeric ketols **6a/b** obtained from esters **5a/b** by enzymatic hydrolysis were subjected to thermal treatment (simultaneous distillation extraction, pH 1), yielding two pairs of diastereomeric 3,4-dihydro-3-oxoedulans (**1a/b** and **1c/d**). The absolute configuration at C-8a was established by NOE experiments. Using on-line coupled multidimensional gas chromatography–mass spectrometry [DB-Wax/octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin] with selected ion monitoring mode, enantiodifferentiation of **1a–d** in a number of natural sources was carried out.

Keywords: *3,4-Dihydro-3-oxoedulans; enantiodifferentiation; MDGC–MS*

INTRODUCTION

Due to its economical importance and the health factors associated with its use, the tobacco plant is one of the most studied plant species. As a result, some 2500 compounds are known as tobacco constituents to date (Wahlberg and Enzell, 1987). Those constituents present in the volatile fractions have received particular attention, and many of them have found commercial use as ingredients in tobacco flavors. One of these compounds is 2,3,5,6,8,8a-hexahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-one (3,4-dihydro-3-oxoedulan) (**1**), a well-known aroma compound of Burley (Demole and Berthet, 1972), Turkish (Schumacher and Vestal, 1974), and Lanka tobacco (Rao et al., 1987). It has also been found among the volatile constituents of purple passion fruit (Winter et al., 1979), *Lycium chinense* M. (Sannai et al., 1983), oak wood (Sefton et al., 1990), and Chardonnay juice (Sefton et al., 1993).

A first synthesis of **1**, whose flavor is described as "oriental tobacco" like with a "woody and ionone" note (Schumacher, 1968; Lloyd et al., 1976), was performed by Schumacher in 1968. However, no attempt was made to establish the absolute configuration of the four stereoisomers of 3,4-dihydro-3-oxoedulan. Since most recently we succeeded in establishing the absolute configuration of isomeric edulans **2** (Schmidt et al., 1995), our interest was focused on the structurally related oxygenated compounds **1a–d**. In this paper our studies on the synthesis of the 3,4-dihydro-3-oxoedulan isomers **1a–d** and their enantiodifferentiation in natural sources are described.



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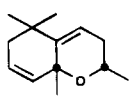

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EXPERIMENTAL PROCEDURES

General. All commercial chemicals used were of analytical grade quality. All solvents employed were of high purity at purchase and were redistilled before use.

Fruits. Fresh ripe purple passion fruit (*Passiflora edulis* Sims) originated from Australia and Africa. Riesling wine and grapevine leaves were from Thüngersheim, Germany. Quince fruit (*Cydonia oblonga* Mill.) was available from the local market. For the isolation of glycosidically bound constituents from fruits and leaves the XAD method (Gunata et al., 1985) with methanol as eluting solvent was used. Enzymatic hydrolyses were carried out using β-D-glucosidase from sweet almond (Serva). The liberated aglycons were extracted with diethyl ether, and after HRGC–MS analysis, subjected to simultaneous distillation extraction (SDE) (Schultz et al., 1977). At first SDE at pH 3.4 was carried out to separate the major part of aglycons; subsequent SDE at pH 1 yielded 3,4-dihydro-3-oxoedulans (**1a–d**) free from interfering peaks.

High-Resolution Gas Chromatography (HRGC). A Hewlett-Packard 5890 gas chromatograph equipped with a J&W fused silica DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and a Carlo Erba Fractovap 4100 gas chromatograph equipped with a J&W fused silica DB-Wax capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) were used. Split injection (1:30) was employed. The temperature program was from 60 to 300 °C at 5 °C/min for DB-5 and from 50 (3 min isothermal) to 240 °C at 4 °C/min for DB-Wax, respectively. The flow rate for the carrier gas was 1.6 mL/min of He, for the makeup gas, 30 mL/min of N_2, and for the detector gases 30 mL/min of H_2 and 300 mL/min of air. The injector temperature was kept at 250 °C and the detector temperature at 280 °C for DB-5; these temperatures for DB-Wax were 220 and 260 °C, respectively. The linear retention index (Ri) is based on a series of *n*-hydrocarbons.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). HRGC–MS was performed with a Varian Aerograph 3300 gas chromatograph by direct coupling to a Finnigan MAT 44 mass spectrometer equipped with a PCDS data system. The same types of columns and the same temperature programs as mentioned above for HRGC analysis were used. Other conditions were as follows: carrier gas flow rate, 2.2 mL/min of He; temperature of ion source and all

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connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.7 mA; injection volumes, 1 μL.

High-Resolution Gas Chromatography–Fourier Transform Infrared Spectroscopy (HRGC–FTIR). HRGC–FTIR analysis was carried out using a Bruker IFS85 system with a wide band MCT detector interfaced with a Carlo Erba Fractovap 2101 AC gas chromatograph equipped with a flame ionization detector. A J&W DB-Wax column (30 m × 0.32 mm i.d.; film thickness 0.5 μm) was used. The temperature program was from 100 to 240 °C at 10 °C/min. The flow rate for the carrier gas (He) was 1.3 mL/min; the light pipe and transfer line were held at 200 °C. Vapor-phase FTIR spectra were recorded from 600 to 4800 cm^{-1} with a resolution of 8 cm^{-1}.

Multidimensional Gas Chromatography–Mass Spectrometry (MDGC–MS). A Siemens Sichromat 2 double-oven gas chromatograph with split injection (250 °C, 1:20) and flame ionization detectors on ovens 1 and 2 (250 °C each) was used. Preseparation was achieved in oven 1 on a J&W DB-Wax fused silica capillary column (25 m × 0.25 mm i.d.; film thickness 0.25 μm). The temperature was programmed from 60 to 240 °C (10 °C/min). A "live" switching device (Schomburg et al., 1984) in oven 1 was used to perform effluent cuts onto column 2 in oven 2 [octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin; 25 m × 0.25 mm i.d.; film thickness 0.25 μm]. The temperature was isothermal at 60 °C for 20 min and then programmed from 60 to 200 °C at 2 °C/min. Two 18 s cuts were carried out. The flow rates for the detector gases were each 30 mL/min of hydrogen and 300 mL/min of air. The MDGC system was directly coupled to a Finnigan MAT 44 quadrupole mass spectrometer using a heated transfer line. The temperature of the ion source and the transfer line was 200 °C. The electron energy was 70 eV and the cathodic current 0.7 mA. Injection volumes of 1 μL were used. Results of analysis were verified by comparison of MDGC–MS (SIM mode) data of authentic optically enriched 3,4-dihydro-3-oxoedulans (**1a–d**).

Chemical Ionization Mass Spectrometry (CI-MS). For CI-MS analyses a Finnigan 8200 mass spectrometer was used (reactant gas NH_3; pressure 0.3 mbar). Positive ions over a range of m/z 70–800 were scanned.

Preparation of Optically Enriched Reference Compounds 1a–d. *(a) Synthesis and Separation of HTA Esters of (E)-3-Oxo-retro-α-ionol 5a/b.* For the preparation of optically enriched 3,4-dihydro-3-oxoedulans (**1a–d**) our method developed for the synthesis of optically pure edulans (**2a–d**) (Schmidt et al., 1995) was modified as outlined in Figure 1, employing HPLC separation of diastereomeric HTA esters of *(E)*-3-oxo-*retro*-α-ionol (**5a/b**). The latter compounds were accessible from α-ionone by oxidation with *tert*-butyl chromate in CCl_4, thus yielding 3-oxo-α-ionone (**3**) (Roberts et al., 1968; Sefton et al., 1989). Treatment of **3** with KOH in MeOH and immediate reduction with $NaBH_4$ yielded 3-oxo-*retro*-α-ionols (**4a/b**) (Schumacher, 1968) (30%) together with the major product **4c**. The *E* isomer (**4a**) was purified by multilayer coil countercurrent chromatography (MLCCC) (Ito et al., 1982). Hexane/ethyl acetate/methanol/water (70:30:14:10) was used as the solvent system, the more dense layer acting as stationary phase (flow rate 2 mL/min, UV detection 254 nm). After preseparation on a preparative MLCCC coil (2.6 mm i.d., 68 m PTFE tubing), repeated analytical MLCCC (1.6 mm i.d., 162 m PTFE tubing) yielded pure isomer **4a**. Retention times were 14.5 (**4a**) and 11.5 h (**4b**) for preparative separation and 13 (**4a**) and 11 h (**4b**) for analytical separation, respectively. Spectral data of **4a** and **4b** have been reported previously (Herderich and Winterhalter, 1991). HTA esters **5a/b** were obtained by adding a solution of 40 mg (1.9×10^{-1} mmol) (*E*)-3-oxo-*retro*-α-ionol (**4a**) in dry CCl_4 (10 mL) to a stirred solution of 97.7 mg (5.8×10^{-1} mmol) of freshly prepared (*R*)-(−)-2-phenylpropionyl chloride (Helmchen and Schmierer, 1976). After 3 days of stirring at room temperature (TLC monitoring), H_2O (20 mL) was added and the water phase extracted with diethyl ether (3 × 20 mL). Combined ether extracts were washed with 0.5 N NaOH (20 mL) and H_2O (20 mL). After drying (Na_2SO_4) and concentration *in vacuo*, the reaction mixture was subjected to flash chromatography on silica gel using diethyl ether/pentane (3:7) as eluent. Final purification

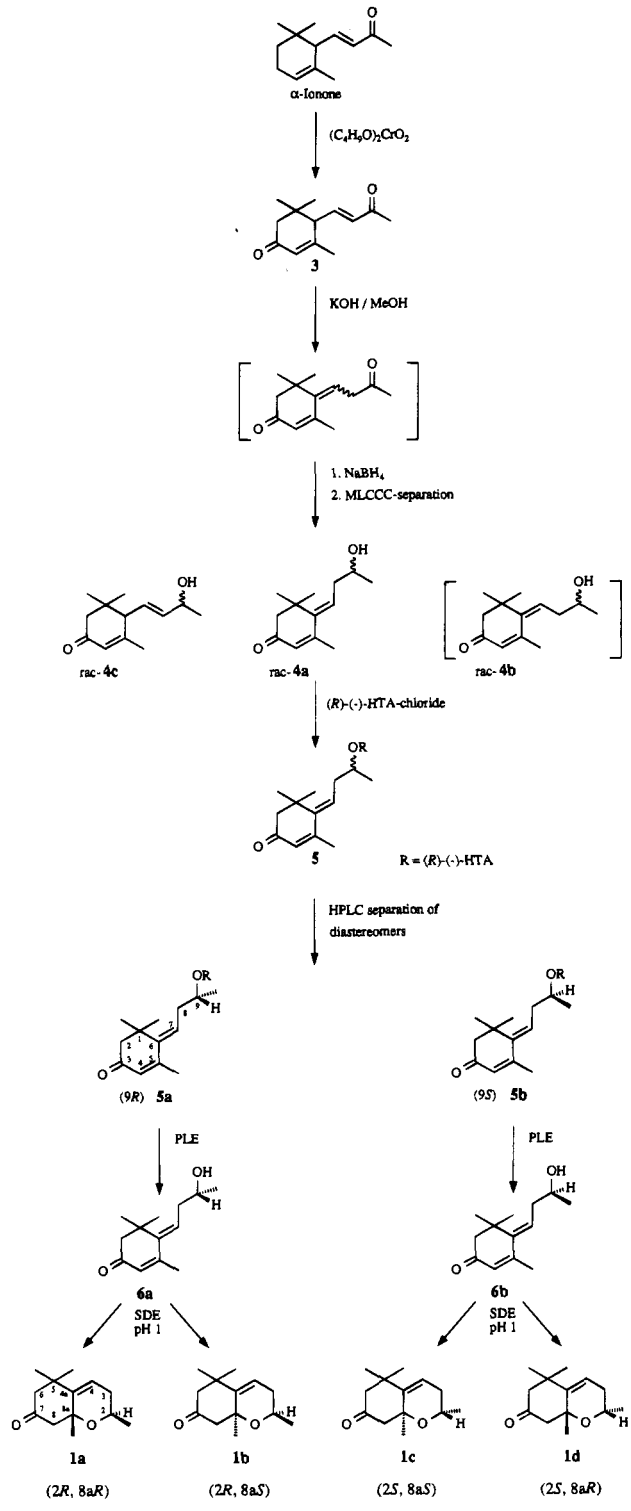

Figure 1. Synthesis scheme of optically enriched isomers **1a–d**.

of diastereomeric esters **5a/b** was obtained by preparative HPLC on a Eurospher Si 100 column (7 μm, 250 × 16 mm; Knauer, Berlin; flow rate 10 mL/min, UV detection 254 nm) using pentane/MTBE (8:2) as eluent. Separated HTA esters **5a** and **5b** showed the following chromatographic and spectral data. First eluting isomer (HPLC retention time 18.7 min) **5a** (5 mg): Ri (DB-5) 2565; CI-MS m/z (%) 358 (100, $[M + NH_4]^+$), 341 (8, $[M + H]^+$), 208 (2), 190 (3), 103 (3); ^1H NMR (200 MHz, $CDCl_3$) δ 1.18 and 1.20 (2 × 3 H, 2 s, $2CH_3$-C1), 1.29 (3 H, d, $J = 6$ Hz, CH_3-C9), 1.48 (3 H, d, $J = 7$ Hz, CH_3-C1′), 1.72 (3 H, d, $J = 1$ Hz, CH_3-C5), 2.26 (2 H, br s, H_2C2), 2.59 (2 H, m, H_2C8), 3.68 (1 H, q, $J = 7$ Hz, HC1′), 5.04 (1 H, m, HC9), 5.64 (1 H, t, $J = 6$ Hz, HC7), 5.81 (1 H, br s, HC4), 7.25 (5 H, m, phenyl-C1′). Second eluting isomer (19.8 min)

5b (5 mg): Ri (DB-5) 2587; CI-MS identical with that of isomer **5a**; ^{1}H NMR δ 1.20 (3 H, d, J = 6 Hz, CH_3-C9), 1.27 (6 H, s, 2CH_3-C1), 1.49 (3 H, d, J = 7 Hz, CH_3-C1′), 1.92 (3 H, d, J = 1 Hz, CH_3-C5), 2.33 (2 H, s, H_2C2), 2.65 (2 H, m, H_2C8), 3.69 (1 H, q, J = 7 Hz, HC1′), 5.04 (1 H, m, HC9), 5.81 (1 H, t, J = 6 Hz, HC7), 5.88 (1 H, br s, HC4), 7.26 (5 H, m, phenyl-C1′).

*(b) Enzymatic Hydrolysis of Ester **5a** and Subsequent Cyclization to Diastereomeric 3,4-Dihydro-3-oxoedulans **1a/b**.* To 5 mg (1.47×10^{-2} mmol) of (9*R*)-HTA ester **5a** (diastereomeric excess, de > 97%; HRGC control) suspended in 5 mL of buffer (pH 7) was added 10 mg of pig liver esterase, and the stirred suspension was incubated overnight at 37 °C. The water phase was extracted with diethyl ether (3 × 20 mL); the combined organic phases were dried over Na_2SO_4 and, after careful concentration (Vigreux column), subjected to flash chromatography on silica gel (100 mm × 10 mm i.d.) using 50 mL of diethyl ether/pentane (1:1) and 50 mL of pure diethyl ether as eluent. The first fraction with product **6a** (yield 50% by HRGC control) was carefully concentrated (Vigreux column) and immediatly subjected to simultaneous distillation/extraction (SDE) treatment at pH 1 (HCl) using a modified Likens–Nickerson head as previously described by Schultz et al. (1977). After drying (Na_2SO_4) of the organic phase, the reaction mixture was carefully concentrated (Vigreux column). Purification by analytical HPLC on either a Eurospher Si 100 column (5 μm, 250 × 4 mm; Knauer, Berlin; flow rate 1 mL/min, UV detection 205 nm) using pentane/MTBE (7:3) as eluent or a Chiralcel OB-H column (250 × 46 mm; Daicel; flow rate 0.5 mL/min, UV detection 205 nm) using hexane/2-propanol (100:2) as eluent yielded a mixture of diastereomeric 3,4-dihydro-3-oxoedulans **1a/b** which were found to be inseparable by HPLC. The second fraction, containing nonhydrolyzed (9*R*)-HTA ester **5a** was repeatedly subjected to enzymatic hydrolysis as described above until complete conversion. Isomer **1a** showed the following chromatographic and spectral data: 2*R*,8a*R* isomer (enantiomeric excess, ee 90%); Ri (DB-5) 1469; Ri (DB-Wax) 1897; UV λ_{max} = 200.5 nm; EIMS data as reported by Sefton et al. (1990); FTIR (vapor phase, ν, cm^{-1}) 2981, 2873, 1732, 1381, 1281, 1234, 1103, 991; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.11 and 1.20 (2 × 3 H, 2s, 2CH_3-C5), 1.20 (3 H, d, J = 6.2 Hz, CH_3-C2), 1.41 (3 H, d, J = 0.9 Hz, CH_3-C8a), 1.96–2.07 (2H, m, H_2C3), 2.21–2.28 and 2.38–2.41 (2 H, m, H_2C8), 2.55 and 2.57 (2 H, 2d, J = 22.6 Hz, H_2C6), 3.54 (1 H, qdd, J = 10.2, 6.1, 3.0 Hz, HC2), 5.90 (1 H, dd, J = 7.3, 2.1 Hz, HC4); ^{13}C NMR (150 MHz, $CDCl_3$) δ 21.3 and 30.5 C10/11 (CH_3), 32.6 C9 (CH_3), 29.7 C12 (CH_3), 33.5 C3 (CH_2), 36.7 C5 (C), 52.9 C6 (CH_2), 54.9 C8 (CH_2), 65.3 C2 (CH), 76.2 C8a (C), 120.4 C4 (CH), 146.2 C4a (C), 209.5 C7 (C). 2*R*,8a*S* isomer **1b**: Ri (DB-5) 1495; Ri (DB-Wax) 1945; UV λ_{max} = 200.5 nm; EIMS data as reported by Sefton et al. (1990); FTIR (vapor phase, ν, cm^{-1}) 2978, 2916, 1732, 1466, 1381, 1284, 1223, 1111, 1065; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.11 and 1.12 (2 × 3 H, 2s, 2CH_3-C5), 1.21 (3 H, d, J = 6.1 Hz, CH_3-C2), 1.47 (3 H, d, J = 0.8 Hz, CH_3-C8a), 2.00–2.05 (1 H, ddd, J = 17.7, 4.5, 4.5 Hz, H_eC3), 2.07–2.12 (1 H, ddd, J = 17.7, 10.2, 2.7 Hz, H_aC3), 2.33 (2 H, s, H_2C8), 2.55 and 2.57 (2 H, 2d, J = 22.6 Hz, H_2-C6), 3.97 (1 H, qdd, J = 10.2, 6.1, 4.5 Hz, HC2), 5.73 (1 H, dd, J = 5.0, 2.7 Hz, HC4); ^{13}C NMR (150 MHz, $CDCl_3$) δ 21.9 and 30.6 C10/11 (CH_3), 27.6 C12 (CH_3), 31.7 C9 (CH_3), 33.5 C3 (CH_2), 36.7 C5 (C), 54.2 C6 (CH_2), 54.5 C8 (CH_2), 63.3 C2 (CH), 75.1 C8a (C), 118.8 C4 (CH), 146.2 C4a (C), 209.2 C7 (C).

*(c) Enzymatic Hydrolysis of Ester **5b** and Subsequent Cyclization to Diastereomeric 3,4-Dihydro-3-oxoedulans **1c/d**.* (9*S*)-HTA ester **5b** (de 74%, HRGC control) was treated as described for the 9*R*-isomer **5a**. SDE treatment yielded diastereomeric 3,4-dihydro-3-oxoedulans **1c/d** (ee 73%), showing identical chromatographic and spectral data as obtained for the corresponding enantiomers **1a/b**. The sense of rotation could only be determinded for the major isomers **1b/d**, i.e. (−) for isomer **1b** and (+) for isomer **1d** (Chiralyzer, IBZ Messtechnik).

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on Bruker SPA31H00 360 MHz, AC 200, WM 400, and SFX 600 instruments with $CDCl_3$ as solvent. Nuclear Overhauser enhancement (NOE) measurements of the carefully degassed samples were performed at ambient temperature by irradiation of the different proton chemical shift frequencies for 4.8 s.

RESULTS AND DISCUSSION

In Figure 1, a scheme of the synthesis of 3,4-dihydro-3-oxoedulan enantiomers **1a–d** is outlined. The benzopyran derivatives **1a–d** were prepared from their presumed natural precursor, 3-oxo-*retro*-α-ionol (**6a/b**), which was available from α-ionone by *tert*-butyl chromate oxidation, rearrangement of the 7,8 double bond with KOH in MeOH, and subsequent reduction of the carbonyl group at C9 with $NaBH_4$. The two geometrical isomers (*E*/*Z*)-3-oxo-*retro*-α-ionols (**4a/b**) were first separated by preparative MLCCC and then by analytical MLCCC. In the first step, the *E* isomer **4a** was preseparated from the major reaction product 3-oxo-α-ionol (**4c**); in the second step, final purification of **4a** was achieved. The *Z* isomer (**4b**) has been found to be inseparable from (**4c**). The racemic keto alcohol **4a** was subsequently esterified with (*R*)-(−)-2-phenylpropionic acid. The resulting diastereomeric esters (**5a** and **5b**) were isolated in pure form by preparative HPLC: The absolute configuration at C9 was established according to the method of Helmchen correlating stereochemistry of chiral secondary alcohols with ^{1}H NMR spectroscopic behavior of their diastereomeric esters prepared from optically pure (*R*)-(−)-2-phenylpropionic acid (Helmchen and Schmierer, 1976; Helmchen, 1974). Comparison of the ^{1}H NMR data of the separated esters **5a** and **5b** showed *inter alia* that the resonance of CH_3-C9 in ester **5a** was downfield shifted, thus indicating *R* configuration at C9. Accordingly, due to the upfield shift for the resonance of CH_3-C9 in esters **5b**, *S* configuration was deduced.

The isomeric keto alcohols **6a** and **6b** were obtained from **5a/b** by enzymatic hydrolysis using pig liver esterase. Since only 50% of the esters were hydrolyzed in a single step, the recovered esters were repeatedly subjected to hydrolysis, thus enabling complete conversion (five cycles) to alcohols **6a** and **6b**. Thermal treatment under SDE conditions was performed at pH 1 using hydrochloric acid. At natural pH (3.4) the yields of **1a–d** were very low; in addition, HCl was found to minimize side reactions. Using sulfuric acid or phosphoric acid, the formation of megastigmatrienones predominated. After SDE, two pairs of diastereomeric 3,4-dihydro-3-oxoedulans (**1a/b**) and (**1c/d**) were obtained in optically enriched form. Separation of the diastereomers was not possible by means of either achiral or chiral HPLC. Nevertheless, the absolute configuration at C8a was established by NOE NMR experiments. Due to the differences in the chemical shift frequencies of the diastereomers **1a/b** and **1c/d** which were formed in a 15:85 ratio (*cis/trans*), assignment of the signals in the NMR spectra was achieved. Thus, e.g., for enantiomer **1b** irradiation of the protons at the methyl group at C8a (1.47 ppm) resulted in a NOE at the C2 methine protone and *vice versa*, implicating 2*R*,8a*S* configuration for this isomer. The enantiomer **1d** showed the same effect in the NOE experiment, thus revealing 2*S*,8a*R* configuration. NOE experiments performed with isomer **1a** showed enhancement for the signal of the methyl protons at C8a while irradiation of the protons at the methyl group at C2 and *vice versa*, implicating the absolute configuration 2*R*,-8a*R* for this isomer. The 2*S*,8a*S* enantiomer **1c** showed the same effect. Concerning the reaction mechanism, it can be assumed that the acid-catalyzed cyclization of

Table 1. Enantiomeric Composition of 3,4-Dihydro-3-oxoedulans 1a–d in Various Natural Sources

source	**1c** (2*S*,8a*S*), %	**1a** (2*R*,8a*R*), %	ee, %	**1d** (2*S*,8a*R*), %	**1b** (2*R*,8a*S*), %	ee, %
bound[a]						
Riesling wine	28	72	44	28	72	44
grapevine leaves	22	78	56	22	78	56
quince	26	74	48	26	74	48
passion fruit (Australia)	94	6	88	94	6	88
passion fruit (Africa)	95	5	90	95	5	90
free						
tobacco	14	86	72	13	87	74

[a] Glycosidically bound precursor liberated by enzymatic hydrolysis and subsequent SDE; 3,4-dihydro-3-oxoedulans were formed by SDE at pH 1.

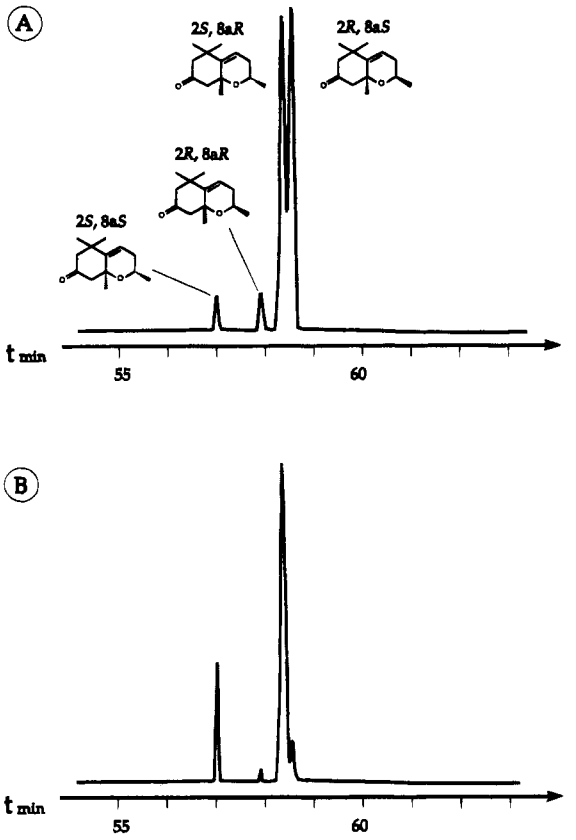

Figure 2. MDGC [DB-Wax/octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin] enantiodifferentiation of 3,4-dihydro-3-oxoedulan isomers **1a–d**: (A) separation of the racemic synthetic mixture and appointed absolute configuration; (B) enantiodifferentiation in a natural sample (passion fruit, Africa).

the keto alcohol (**6a/b**) and the equilibration of the 3,4-dihydro-3-oxoedulans take place through the common pentadienyl carbonium ion.

Using GC sniffing, the 3,4-dihydro-3-oxoedulans exhibited only weak odor intensities. In the mixture of 2*S*,8a*S* and 2*S*,8a*R* isomers **1c/1d** (organic solution, ratio 15:85, by HRGC), a camphoraceous note dominated, whereas in the mixture of 2*R*,8a*R* and 2*R*,a*S* isomers **1a/1b** (organic solution, ratio 15:85, by HRGC), a weak tobacco note was detectable.

Using on-line coupled multidimensional gas chromatography–mass spectrometry (MDGC–MS) (Bernreuther and Schreier, 1991) with SIM mode, enantiodifferentiation of **1a–d** in natural sources was carried out. The results of these studies are summarized in Table 1. The order of elution of the four 3,4-dihydro-3-oxoedulan isomers **1a–d** on a chiral column [octakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin] was found to be **1c** (2*S*,8a*S*), **1a** (2*R*,8a*R*), **1d** (2*S*,8a*R*), and **1b** (2*R*,8a*S*). As a representative example in Figure 2 the MDGC separation of **1a–d** in an extract of purple passion fruit is outlined. In this fruit the 2*S* isomers prevailed, whereas in Riesling wine, grapevine leaves, quince fruit, and tobacco the 2*R* isomers were predominant. Since the formation of **1a–d** proceeds via the presumed precursor **6a/b**, these results also imply the same configuration at the C9 chiral center for the precursor compound.

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LITERATURE CITED

Bernreuther, A.; Schreier, P. Multidimensional gas chromatography-mass spectrometry: a powerful tool for the direct chiral evaluation of aroma compounds in plant tissues. II. Linalool in essential oils and fruits. *Phytochem. Anal.* **1991**, *2*, 167–170.

Demole, E.; Berthet, D. Volatile to medium-volatile constituents (b.p. ≤ 84°/0.001 Torr); a chemical study of Burley tobacco flavour (*Nicotiana tabacum* L.). *Helv. Chim. Acta* **1972**, *55*, 1866–1882.

Gunata, Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **1985**, *331*, 83–90.

Helmchen, G. New method for the determination of the absolute configuration of chiral secondary alcohols and amines. NMR spectroscopy of diastereoisomeric esters and amides of α-phenylbutyric and hydratropic acid. *Tetrahedron Lett.* **1974**, *16*, 1527–1530.

Helmchen, G.; Schmierer, R. Determination of the absolute configuration of chiral thiols by proton NMR spectroscopy of diastereomeric thiol esters. *Angew. Chem.* **1976**, *88*, 770–771.

Herderich, M.; Winterhalter, P. 3-Hydroxy-retro-α-ionol: a natural precursor of isomeric edulans in purple passion fruit (*Passiflora edulis* Sims). *J. Agric. Food Chem.* **1991**, *39*, 1270–1274.

Ito, Y.; Sandlin, J.; Bowers, W. G. High-speed preparative countercurrent chromatography with a coil planet centrifuge, *J. Chromatogr.* **1982**, *244*, 247–258.

Lloyd, R. A.; Miller, C. W.; Roberts, D. L.; Giles, J. A.; Dickerson, J. P.; Nelson, N. H.; Rix, C. E.; Ayers, P. H. Fluecured tobacco flavor. I. Essence and essential oil components. *Tob. Sci.* **1976**, *20*, 125–133.

Rao, B. V. K.; Murthy, P. S. N.; Chakraborty, M. K.; Fujimori, T. Chemical studies on Lanka tobacco and its smoke. 3. Essential oils. *Tob. Res.* **1987**, *13*, 14–26.

Roberts, D. L.; Heckman, R. A.; Hege, B. P.; Bellin, S. A. Synthesis of (*RS*)-abscisic acid. *J. Org. Chem.* **1968**, *33*, 3566–3569.

Sannai, A.; Fujimori, T.; Kato, K. Neutral volatile components of Kukoshi (*Lycium chinense* M.). *Agric. Biol. Chem.* **1983**, *47*, 2397–2399.

Schmidt, G.; Full, G.; Winterhalter, P.; Schreier, P. Synthesis and enantiodifferentiation of isomeric 3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-2*H*-1-benzopyrans (edulans I and II). *J. Agric. Food Chem.* **1995**, *43*, 185–188.

Schomburg, G.; Husmann, H.; Podmaniczky, L.; Weeke, F. Coupled gas chromatographic methods for separation, identification, and quantitative analysis of complex mixtures: MDGC, GC-IR, LC-GC. In *Analysis of Volatiles*; Schreier, P., Ed.; de Gruyter: Berlin, 1984; pp 121–150.

Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. Isolation of volatile compounds from a model system. *J. Agric. Food Chem.* **1977**, *25*, 446–449.

Schumacher, J. N. Flavoring tobacco with a benzopyran (Reynolds Tobacco Co.). U.S. Pat. 3 389 706, 1968; *Chem. Abstr.* **1968**, 41817q.

Schumacher, J. N.; Vestal, L. Isolation and identification of smoke components of Turkish tobacco. *Tob. Sci.* **1974**, *18*, 43–48.

Sefton, M. A.; Skouroumounis, G. K.; Massy-Westropp, R. A.; Williams, P. J. Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Aust. J. Chem.* **1989**, *42*, 2071–2084.

Sefton, M. A.; Francis, I. L.; Williams, P. J. Volatile norisoprenoid compounds as constituents of oak woods used in wine and spirit maturation. *J. Agric. Food Chem.* **1990**, *38*, 2045–2049.

Sefton, M. A.; Francis, L. I.; Williams, P. J. The volatile composition of Chardonnay juices: a study by flavor precursor analysis. *Am. J. Enol. Vitic.* **1993**, *44*, 359–369.

Wahlberg, I.; Enzell, C. R. Tobacco isoprenoids. *Nat. Prod. Rep.* **1987**, *4*, 237–276.

Winter, M.; Näf, F.; Furrer, A.; Pickenhagen, W.; Giersch, W.; Meister, A.; Willhalm, B.; Thommen, W.; Ohloff, G. (Z)-4,7-Octadienoic acid ethyl ester und (Z)-butyric acid 3,5-hexadienyl ester, two new aroma compounds from the red passion fruit. *Helv. Chim. Acta* **1979**, *62*, 135–139.

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