van Straten, S.; Maarse, H. In *Volatile Compounds in Food Qualitative Data*, 5th ed.; Division for Nutrition and Food Research TNO: Zeist, 1983; Section 81.2-81.5.
Winter, M.; Malet, G.; Pfeiffer, M.; Demole, E. "Structure d'Une Lactone Odorante Présente dans l'Essence de Jasmin (*Jasminum grandiflorum* L.)". *Helv. Chim. Acta* **1962**, *45*, 1250–1255.
Yabumoto, K.; Ingraham, D. F.; Jennings, W. G. "The Overload Phenomenon in Gas Chromatography". *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1980**, *3*, 248–252.
Yamanishi, T.; Kawatsu, M.; Yokoyama, T.; Nakatani, Y. "Methyl Jasmonate and Lactones Including Jasmine Lactone in Ceylon Tea". *Agric. Biol. Chem.* **1973**, *37*, 1075–1078.

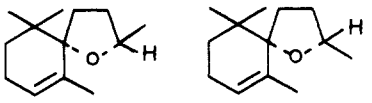
Received for review February 24, 1987. Revised manuscript received October 7, 1987. Accepted October 26, 1987. Names of products are included for the benefit of the reader and do not imply endorsement by USDA.

4-Hydroxy-7,8-dihydro-β-ionol: Natural Precursor of Theaspiranes in Quince Fruit (*Cydonia oblonga*, Mill.)

Peter Winterhalter and Peter Schreier*

In a polar fraction of an extract obtained from quince fruit juice (*Cydonia oblonga*, Mill.) by solvent extraction (pentane–dichloromethane, 2:1) and subsequent LC separation on silica gel, capillary gas chromatography (HRGC), and coupled HRGC techniques, i.e. on-line mass spectrometry (HRGC–MS) and Fourier transform infrared spectroscopy (HRGC–FTIR), the occurrence of 4-hydroxy-7,8-dihydro-β-ionol was revealed. The identification was verified by comparison of HRGC, HRGC–MS, and HRGC–FTIR data of the natural product with those of an authentic reference compound synthesized from 4-oxo-β-ionol. Model reactions carried out with 4-hydroxy-7,8-dihydro-β-ionol under high-vacuum distillation/extraction conditions (40 °C, 5 h) showed the easy formation of theaspiranes at pH 3.5. The mechanism of theaspirane formation from 4-hydroxy-7,8-dihydro-β-ionol can be considered as "prototropic dehydration of an allyl-1,6-diol" as previously described for monoterpene diols.

The diastereoisomeric spiro ethers theaspirane **1A** and **1B** have been found in raspberries (Winter and Enggist, 1971), yellow passion fruit (Winter and Klöti, 1972), black tea aroma (Renold et al., 1974), grapes (Schreier et al., 1976), Osmanthus absolute (Kaiser and Lamparsky, 1978), guava (Idstein and Schreier, 1985), black chokeberry (*Aronia melanocarpa* Ell.) (Hirvi and Honkanen, 1985), and quince fruit (Tsuneya et al., 1983; Winterhalter et al., 1987). Ether **1A** possesses a fresh green and woody–



1A **1B**

campherous odor, characteristic of many oxygenated bicyclic monoterpenes, whereas this note almost becomes naphthalene-like in the diastereoisomeric ether **1B** (Skorianetz et al., 1975; Ohloff, 1978). The theaspiranes are widely used in the flavor industry. In tobacco only 5 ppb suffice to reach the optimum flavor, and in order to round off fruit flavors, 1 ppb in the finished product is sufficient (Naegeli, 1977). Starting from ionone derivatives, several syntheses for **1A/1B** have been described (Nakatani and Yamanishi, 1969; Ina et al., 1972; Naegeli, 1977; Etoh et al., 1980).

In our recent work on quince flavor (Winterhalter et al., 1987) it was demonstrated that **1A/1B** were obviously not original volatiles but were formed at natural pH of the fruit pulp, i.e. under acidic conditions (pH 3.5–3.8) from a labile precursor. In this paper, we report the isolation and identification of this natural precursor of **1A/1B**.

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, D-8700 Würzburg, West Germany.

EXPERIMENTAL SECTION

Fruits. Fresh ripe quince fruits (*Cydonia oblonga*, Mill.) were available from the local market.

Sample Preparation. Freshly prepared juice was obtained from 10 kg of cut fruits (seeds removed) after crushing and pressing (Hafico press). The juice (8.5 kg) was diluted with distilled water (1:1, v/v) and subjected to solvent extraction using pentane–dichloromethane (2:1) over 24 h (Drawert and Rapp, 1968). The extract was dried over anhydrous sodium sulfate and carefully concentrated to 1 mL on a Vigreux column (45 °C). The extract was fractionated on silica gel 60 (Merck), activity grade II, by applying a pentane–diethyl ether gradient. Cooled (11–13 °C) glass columns, 2 cm (i.d.) × 40 cm, were used. The elution rate was 120 mL/h, and seven fractions were separated: fraction I, 500 mL of pentane–diethyl ether (4:1, v/v); fraction II, 500 mL of pentane–diethyl ether (1:1, v/v); fraction III, 500 mL of pentane–diethyl ether (1:4, v/v); fractions IV.1–IV.4, each 250 mL of diethyl ether. All eluates were dried over anhydrous sodium sulfate and concentrated to 0.5 mL (for HRGC and HRGC–MS study) and to 0.1 mL (for HRGC–FTIR analysis), respectively.

Synthesis of *retro*-α-Ionols. *a. Preparation of retro-α-Ionones.* A solution of 17.2 g of β-ionone in 80 mL of dimethyl sulfoxide (cooled to −10 °C) was treated with 6 g of powdered sodium methoxide. After being stirred for 1 h at room temperature, the mixture was poured into ice and extracted twice with 250 mL of pentane. After the mixture was dried over anhydrous sodium sulfate and solvent removed, distillation gave 11 g of the isomeric *retro*-α-ionones (1:4 isomers about the exocyclic double bond): ^{1}H NMR (60 MHz, $CDCl_3$), in close agreement with published data (Rosenberger et al., 1980); FTIR (vapor phase, ν, cm^{-1}) 3006, 2965, 2929, 1732, 1452, 1359, 1205, 1156, 871; EIMS *m/z* (%) isomer 1 (R_t 1437) 43 (100) 55(28) 69 (48) 79 (23) 93 (52) 107 (51) 121 (52) 134 (5) 149 (28) 159 (3) 174 (2) 177 (2) 192 (13, M^+); EIMS *m/z* (%)

0021-8561/88/1436-0560$01.50/0

Table I. Spectral Data of 4-Hydroxy-7,8-dihydro-β-ionol (2) (R_t 1660)

UV (λ_{max}, EtOH): 211.4 nm
MS (EI, 70 eV) *m/z* (%): 43 (44), 55 (24), 67 (10), 82 (34), 96 (35), 109 (24), 119 (23), 138 (100), 150 (1), 161 (2), 176 (1), 194 (4, M − H_2O)
FTIR (vapor phase, ν, cm^{-1}): 3650 (sec-OH), 2975, 2931, 2878, 1458, 1382, (*gem* dimethyl), 1086 and 1019 (C–O)
^1H NMR (200 MHz, $CDCl_3$, TMS): δ 0.97 and 1.04 (each 3 H, 2 s; 2 CH_3Cl), 1.22 (3 H, d, *J* = 6.2 Hz; CH_3-C9), 1.74 (3 H, s; CH_3-C5), 3.81 (1 H, sext, *J* = 6.2 Hz; H-C9), 3.89 (1 H, t, *J* = 4.3 Hz; H-C4)

isomer 2 (R_t 1478) 43 (100) 55 (29) 69 (49) 79 (20) 93 (51) 107 (43) 121 (48) 134 (5) 149 (34) 159 (3) 174 (2) 177 (2) 192 (15, M^+).

b. Preparation of retro-α-Ionols. To a solution of 1 g of isomeric *retro*-α-ionones in 50 mL of diethyl ether was added 250 mg of $LiAlH_4$ under cooling and the mixture stirred at room temperature for 1 h. After addition of ice–water the mixture was extracted three times with 100 mL of diethyl ether. Drying over anhydrous sodium sulfate and removal of the solvent afforded 0.9 g of retro-α-ionols: ^1H NMR and IR data, see Schulte-Elte et al. (1978); EIMS *m/z* (%) isomer 1 (R_t 1459) 41 (78) 55 (46) 69 (57) 79 (54) 93 (83) 107 (87) 121 (80) 135 (100) 150 (52) 161 (3) 194 (28, M^+); EIMS *m/z* (%) isomer 2 (R_t 1506) 41 (82) 55 (50) 69 (61) 79 (56) 93 (77) 107 (85) 121 (79) 135 (100) 150 (53) 161 (3) 194 (30, M^+).

c. Acid Treatment of retro-α-Ionols. A 100-mg portion of *retro*-α-ionols in 200 mL of distilled water (acidified with 0.5 g of $KHSO_4$) was subjected to simultaneous distillation/extraction for 2 h with 60 mL of pentane–diethyl ether (1:1) (Schultz et al., 1977). After being dried over anhydrous sodium sulfate, the sample was analyzed by HRGC, HRGC–MS, and HRGC–FTIR.

Synthesis of 4-Hydroxy-7,8-dihydro-β-ionol (2). To a solution of 0.73 g of 4-oxo-β-ionol (**5**) in 20 mL of methanol was added 50 mg of Pd on $BaSO_4$ (5%) and the resultant mixture hydrogenated at room temperature and atmospheric pressure for 30 min. After separation of the catalyst by filtration, quantitative HRGC analysis revealed 80% 4-oxo-7,8-dihydro-β-ionol (**6**) together with 20% **5**. Spectral data of **6** were identical with those published by Kaiser and Lamparsky (1978). After solvent evaporation under vacuum the residue was resolved in 30 mL of dry diethyl ether. To this solution was added 200 mg of $LiAlH_4$ under cooling and the resultant mixture stirred at room temperature for 1 h. After addition of ice–water and extraction with diethyl ether 0.7 g of **2** was obtained (80%; remaining 15% 4-hydroxy-β-ionol and 5% **1A/1B**). After LC purification on silica gel using a pentane–diethyl ether gradient, **2** was obtained in pure form. Chromatographic and spectral data of **2** are outlined in Table I.

Acetylation of 2. To a solution of 10 mg of **2** in 0.5 mL of toluene were added 0.1 mL of pyridine and 0.1 mL of acetic anhydride, and the mixture was stirred at room temperature for 24 h. HRGC–MS and HRGC–FTIR analyses revealed the following data for 4-hydroxy-7,8-dihydro-β-ionyldiacetate (R_t 1893): EIMS *m/z* (%) 43 (100) 55 (22) 91 (10) 105 (15) 119 (48) 121 (18) 134 (7) 152 (16) 161 (9) 176 (3) 194 (1) 236 (M − HO − Ac, 1); FTIR (vapor phase, ν, cm^{-1}) 1756, 1236.

In like manner, silica gel fraction IV.2 from quince (cf. above) was acetylated. The HRGC and spectral data of 4-hydroxy-7,8-dihydro-β-ionyldiacetate were identical with those of acetylated constituent from quince fruit.

High-Vacuum Distillation of 2 at Different pH Values. Five milliliters of a solution of **2** in diethyl ether (1 mg/mL) and 1 mL of standard solution (10 mg/mL of 1-heptanol in diethyl ether) were added to 2 L of distilled water (pH 7.0), and the resultant mixture was high-vacuum distilled at 40 °C for 5 h. The residue was then acidified with 0.1 N HCl to pH 3.5 and, after addition of 1 mL of standard solution, again high-vacuum distilled. The distillates were extracted for 20 h using pentane–dichloromethane (2:1), dried over anhydrous sodium sulfate, concentrated on a Vigreux column, and subjected to HRGC and HRGC–MS analysis.

Capillary Gas Chromatography (HRGC). A Carlo Erba Fractovap 4160 gas chromatograph with FID equipped with a J&W fused silica DB 5 capillary column (30 m, 0.259 mm (i.d.), df = 0.25 μm) was used. Split injection (1:50) was employed. The temperature program was from 60 to 300 °C at 5 °C/min. The flow rates for the carrier gas were 2.5 mL/min He, for the makeup gas 30 mL/min N_2, and for the detector gases 30 mL/min H_2 and 300 mL/min air, respectively. The detector temperature was kept at 250 °C. Volumes of 1 μL were injected.

Results of qualitative analyses were verified by comparison of HRGC retention, mass spectral, and FTIR vapor-phase data with those of authentic reference substances. Quantitative HRGC determinations were carried out by standard controlled calculations using a Hewlett-Packard 3388 A laboratory data system.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 1440 gas chromatograph equipped with a Carlo Erba water-cooled on-column injection system was combined by direct coupling to a Finnigan MAT 44 mass spectrometer. The same type of column as mentioned above for HRGC analysis was used. The conditions were as follows: temperature, isothermal for 2.5 min at 60 °C and then from 60 to 240 °C at 5 °C/min; carrier gas flow rate, 2.5 mL/min He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA. Volumes of 0.5 μL were used.

Capillary Gas Chromatography–Fourier Transform Infrared Spectroscopy (HRGC–FTIR). HRGC–FTIR analysis was carried out with a Nicolet 20 SXB system interfaced by a Dani 6500 gas chromatograph equipped with FID. The same type of column as mentioned before was used. Total sample injection mode employing programmed temperature vaporization (PTV) (40–240 °C, 0.1 min) was performed. The temperature program was 1 min isothermal at 60 °C and then from 60 to 250 °C at 4 °C/min. Light pipe and transfer line were held at 250 °C; He (2.5 mL/min) was employed as carrier gas. Vapor phase FTIR spectra were recorded from 600 to 4000 cm^{-1} with a resolution of 8 cm^{-1}.

RESULTS AND DISCUSSION

According to the synthetic work carried out on theaspiranes **1A/1B** by Schulte-Elte et al. (1978), 7-hydroxy-7,8-dihydro-β-ionol could be considered as natural precursor of **1A/1B**. Compounds **1A/1B** were formed from this diol on strong-acid treatment, whereas isomeric dihydroedulanes and *retro*-α-ionols were obtained as unstable byproducts on mild-acid treatment (Winterhalter et al., 1987). However, due to the results of our present studies on the behavior of *retro*-α-ionols under acidic conditions, which only led to 35% degradation of the educts, the precursor role of 7-hydroxy-7,8-dihydro-β-ionol seemed to be unlikely. After capillary gas chromatographic (HRGC) and coupled HRGC, i.e. on-line mass spectrometric (HRGC–MS) and Fourier transform infrared spectroscopic (HRGC–FTIR) analysis of extracts obtained from quince fruit juice by pentane–dichloromethane (2:1) extraction and subsequent silica gel LC separation, 7-

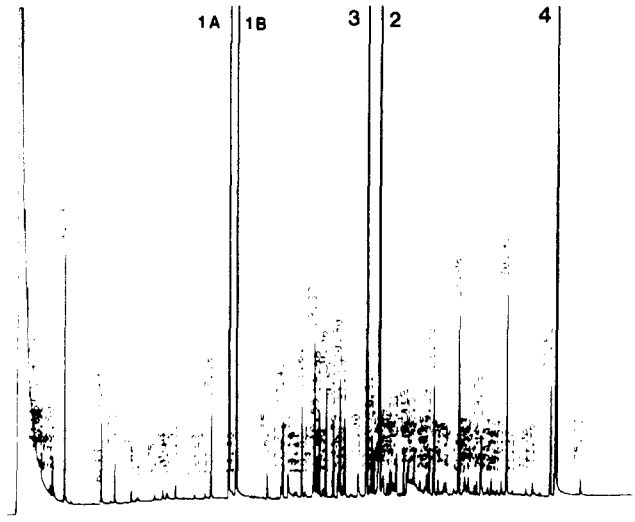
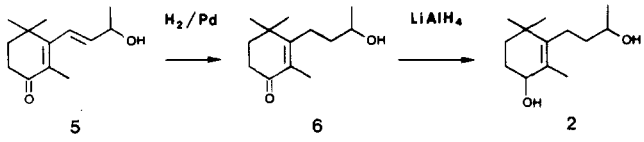


Figure 1. HRGC separation of fraction IV.2 of quince volatiles on a J&W 30 m × 0.259 mm (i.d.) DB 5 fused silica WCOT capillary column, df = 0.25 μm: **1A/1B**, theaspiranes A and B; **2**, 4-hydroxy-7,8-dihydro-β-ionol; **3**, 4-hydroxy-β-ionol; **4**, unknown; not further studied.



5 6 2

Figure 2. Synthesis of 4-hydroxy-7,8-dihydro-β-ionol (**2**) from 4-oxo-β-ionol (**5**) by H_2/Pd and $LiAlH_4$ hydrogenation.

hydroxy-7,8-dihydro-β-ionol could be excluded as natural precursor of **1A/1B**. Neither this compound nor dihydroedulanes or retro-α-ionols were detected. Instead, 4-hydroxy-β-ionol (**3**) was identified as one of the major constituents of the polar silica gel fraction IV.2 (Figure 1). In addition, substance **2** was separated by HRGC, which exhibited a mass spectrum very similar to that of **1A/1B**. Surprisingly, **1A/1B** were also found in this polar fraction, indicating the occurrence of a labile precursor. Since compound **4** (Figure 1), which eluted in pure form in silica gel fraction IV.4, did not lead to any formation of volatiles at pH 3.5 under simultaneous distillation/extraction conditions (Schultz et al., 1977), it was not further investigated and our interest was focused on compound **2**.

Additional information about the structure of **2** was available from the vapor-phase FTIR data, indicating the presence of OH group (3650 cm^{-1}) and the lack of C–O ether bond (1080 cm^{-1}) as well as trans-substituted double bond (970 cm^{-1}). From mass spectral and vapor-phase FTIR data structure **2** was proposed. Its synthesis was performed as outlined in Figure 2. Partial hydrogenation of 4-oxo-β-ionol (**5**) delivered 4-oxo-7,8-dihydro-β-ionol (**6**), from which $LiAlH_4$ reduction easily led to **2**, which has not been described as yet. The synthesized diol **2** showed chromatographic and spectral data (Table I) similar to those of the unknown natural constituent of quince (Figure 1).

Model reactions carried out with **2** in analogy to sample preparation of quince volatiles, i.e. under high-vacuum distillation/extraction conditions (40 °C, 5 h) at pH 3.5 and 7.0 (Winterhalter et al., 1987), revealed the easy formation of **1A/1B** from **2** at pH 3.5. About 50% of **2** was transformed to **1A/1B** under acidic conditions, whereas at pH 7.0 only traces of the spiro ethers were formed. The mechanism of theaspirane formation from the natural precursor **2** can be considered as "prototropic dehydration of an allyl-1,6-diol" as previously described by Ohloff et al. (1964) for monoterpene diols giving rise to tetrahydrofuran derivatives.

ACKNOWLEDGMENT

C. Kahre's skillful HRGC–FTIR analyses are gratefully acknowledged. We thank Dr. E. J. Brunke, Dragoco, Holzminden, and Dr. D. Lamparsky, Givaudan, Dübendorf, for providing several reference compounds as well as the Deutsche Forschungsgemeinschaft, Bonn, for financial support.

Registry No. **1A**, 66537-39-1; **1B**, 66537-40-4; **2**, 113110-02-4; **2** (diacetate), 113110-03-5; **3**, 27185-80-4; **5**, 27185-78-0; **6**, 27185-79-1; (*E*)-*retro*-α-ionone, 55093-41-9; (*Z*)-*retro*-α-ionone, 76826-76-1; (*E*)-*retro*-α-ionol, 55093-46-4; (*Z*)-retro-α-ionol, 55093-47-5; β-ionone, 79-77-6.

LITERATURE CITED

Drawert, F.; Rapp, A. *Chromatographia* **1968**, *1*, 446.
Etoh, H.; Ina, K.; Iguchi, M. *Agric. Biol. Chem.* **1980**, *44*, 2871.
Hirvi, T.; Honkanen, E. *J. Sci. Food Agric.* **1985**, *36*, 808.
Idstein, H.; Schreier, P. *J. Agric. Food Chem.* **1985**, *33*, 138.
Ina, K.; Takano, T.; Imai, Y.; Sakato, Y. *Agric. Biol. Chem.* **1972**, *36*, 1033.
Kaiser, R.; Lamparsky, D. *Helv. Chim. Acta* **1978**, *61*, 2328.
Kaiser, R.; Kappeler, A.; Lamparsky, D. *Helv. Chim. Acta* **1978**, *61*, 387.
Nakatani, Y.; Yamanishi, T. *Tetrahedron Lett.* **1969**, 1995.
Naegeli, P. Ger. Offen. 2610238, 1976; *Chem. Abstr.* **1977**, *86*, 95876a.
Ohloff, G. *Perfum. Flavor.* **1978**, *3*, 11.
Ohloff, G.; Schulte-Elte, K. H.; Willhalm, B. *Helv. Chim. Acta* **1964**, *47*, 602.
Renold, W.; Näf-Müller, R.; Keller, U.; Willhalm, B.; Ohloff, G. *Helv. Chim. Acta* **1974**, *57*, 1301.
Rosenberger, M.; Jackson, W.; Saucy, G. *Helv. Chim. Acta* **1980**, *63*, 1665.
Skorianetz, W.; Renold, W.; Ohloff, G.; Schulte-Elte, K. H. Ger. Offen. 2504618, 1975; *Chem. Abstr.* **1976**, *84*, 43818g.
Schreier, P.; Drawert, F.; Junker, A. *J. Agric. Food Chem.* **1976**, *24*, 331.
Schulte-Elte, K. H.; Gautschi, F.; Renold, W.; Hauser, A.; Fankhauser, P.; Limacher, J.; Ohloff, G. *Helv. Chim. Acta* **1978**, *61*, 1125.
Schultz, T. H.; Flath, R. A.; Mon, R.; Eggling, S. B.; Teranishi, R. *J. Agric. Food Chem.* **1977**, *25*, 446.
Tsuneya, T.; Ishihara, M.; Shiota, H.; Shiga, M. *Agric. Biol. Chem.* **1983**, *47*, 2495.
Winter, M.; Enggist, P. *Helv. Chim. Acta* **1971**, *54*, 1891.
Winter, M.; Klöti, R. *Helv. Chim. Acta* **1972**, *55*, 1916.
Winterhalter, P.; Lander, V.; Schreier, P. *J. Agric. Food Chem.* **1987**, *35*, 335.

Received for review May 18, 1987. Accepted October 7, 1987.