Identification of Novel Glycosidic Aroma Precursors in Saffron (*Crocus sativus* L.)

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The methanolic extract of saffron was separated with the aid of multilayer coil countercurrent chromatography. After purification by HPLC, the following seven novel carotenoid metabolites were identified on the basis of their spectral (UV, FTIR, MS, NMR, CD) data: (4*R*)-4-hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde *O*-*β*-D-gentiobioside (**1**), (4*R*)-4-hydroxy-2,6,6-trimethylcyclohex-1-enecarboxylic acid *O*-*β*-D-glucopyranoside (**2**), 6-hydroxy-3-(hydroxymethyl)-2,4,4-trimethylcyclohexa-2,5-dienone 6-*O*-*β*-D-glucopyranoside (**3**), (2*Z*)-3-methylpent-2-enedioic acid 1-[1-(2,4,4-trimethyl-3,6-dioxocyclohexenyloxy)-*O*-*β*-D-glucopyranosid-6-yl] ester (**4**), (5*S*)-5-hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3*H*-isobenzofuran-1-one *O*-*β*-D-glucopyranoside (**5**), (1*R*,5*S*,6*R*)-5-(hydroxymethyl)-4,4,6-trimethyl-7-oxabicyclo[4.1.0]heptan-2-one *O*-*β*-D-glucopyranoside (**6**), and (1*R*)- 3,5,5-trimethylcyclohex-3-enol *O*-*β*-D-glucopyranoside (**7**).

Keywords: *Carotenoid metabolites; glycosides; aroma precursor; multilayer coil countercurrent chromatography; saffron; Crocus sativus*

INTRODUCTION

In a recent study, Tarantilis and Polissiou (1997) investigated the influence of different isolation techniques on the composition of the volatile fraction of saffron. By using either steam distillation (SD), micro-simultaneous steam distillation−solvent extraction (MSDE), or vacuum headspace (VHS) method, the authors obtained aroma isolates from saffron that exhibited clear differences in their compositional data. For example, by using the vigorous isolation conditions of SD, significantly more higher boiling compounds were detected compared to those found by VHS. The authors assumed that during the isolation procedure oxidative processes take place, converting, for example, safranal (2,6,6-trimethylcyclohexa-1,3-dienecarbaldehyde, **14**), the major aroma volatile of saffron, into the corresponding carboxylic acid **20**. Moreover, a degradation of saffron carotenoids during the isolation process was considered as being likely, thus adding additional breakdown products to the volatile fraction of the spice. In continuation of our work on glycosidic constituents of saffron (Straubinger et al., 1997), we now report the isolation of seven novel glycosidic constituents **1**−**7** (Figure 1) from a methanolic extract of saffron and discuss their precursor function with regard to the formation of the above-mentioned aroma compounds.

EXPERIMENTAL PROCEDURES

General Procedures. Preparation of solvent extracts (petroleum ether, diethyl ether, methanol) from saffron and details of preseparation of the methanolic extract using multilayer coil countercurrent chromatography (MLCCC) have been published earlier (Straubinger et al., 1997). A commercially available saffron sample (type: electus pulvis; Galke, Gittelde, Germany; 8.8 g) has been used.

Isolation of Glycosidic Constituents. After preseparation by MLCCC, the methanolic extract was acetylated (Ac_2O/pyridine) and further fractionated using preparative and analytical HPLC (*n*-hexane/MTBE gradients). In this way, seven glycosidic carotenoid metabolites were obtained in the pure form. Spectroscopic data for isolated compounds **1**−**7** follow.

*(4R)-4-Hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde O-β-D-gentiobioside (**1**)* was isolated as heptaacetate (**1a**): 6.6 mg; $[\alpha]_D$ −17.5° (*c* 0.08% in MeOH); UV (MeOH) λ_{max} 246 nm; CD (*c* 0.002% in MeOH) $[\theta]_{216}$ −7882, $[\theta]_{244}$ −16979, $[\theta]_{337}$ +2971; IR (NaCl) ν 2958, 2875, 1755 (C=O), 1671 (C=O), 1434, 1374, 1222, 1171, 1038, 907, 756 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m*/*z* 804, indicating a molecular mass of 786 ($C_{36}H_{50}O_{19}$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.26 and 1.27 (2 × 3H, 2s, 2 Me-C6), 1.55 (1 H, dd, J = 12.5, 12.0 Hz, $H5_a$), 1.81 (1H, ddd, J = 12.5, 3.5, 2.0 Hz, $H5_b$), 1.99−2.09 (7 × 3H, 7s, 7 acetates), 2.11 (3H, br s, Me-C2), 2.20 (1H, dd, J = 18.0, 9.0 Hz, $H3_a$), 2.49 (1H, ddd, J = 18.0, 5.5, 2.0 Hz, $H3_b$), 3.68 (1H, ddd, J = 10.0, 5.0, 2.5 Hz, H5″), 3.69 (1H, dd, J = 10.5, 5.0 Hz, $H6'_a$), 3.70 (1H, ddd, J = 10.0, 5.0, 2.0 Hz, H5′), 3.84 (1H, dd, J = 10.5, 2.0 Hz, $H6'_b$), 3.99 (1H, dddd, J = 12.0, 9.0, 5.5, 3.5 Hz, H4), 4.12 (1H, dd, J = 12.0, 2.5 Hz, $H6''_a$), 4.26 (1H, dd, J = 12.0, 5.0 Hz, $H6''_b$), 4.57 (1H, d, J = 8.0 Hz, H1″), 4.63 (1H, d, J = 8.0 Hz, H1′), 4.92 (1H, dd, J = 10.0, 9.5 Hz, H4′), 4.93 (1H, dd, J = 9.5, 8.0 Hz, H2′), 4.97 (1H, dd, J = 9.5, 8.0 Hz, H2″), 5.05 (1H, dd, J = 10.0, 9.5 Hz, H4″), 5.17 (1H, dd, J = 9.5, 9.5 Hz, H3″), 5.20 (1H, dd, J = 9.5, 9.5 Hz, H3′), 10.11 (1H, s, C*H*O−C1); ^{13}C NMR (91 MHz, $CDCl_3$) δ 19.2 (Me-C2), 27.9 and 28.7 (2 Me-C6), 35.4 (C6), 41.0 (C3), 46.6 (C5), 61.9 (C6″), 68.0 (C6′), 68.4 (C4″), 69.1 (C4′), 71.1 (C2″), 71.4 (C4), 71.5 (C2′), 72.1 (C5″), 72.8 (C3″), 72.9 (C3′), 73.4 (C5′), 98.9 (C1′), 100.6 (C1″), 140.3 (C1), 150.6 (C2), 191.4 (CHO−C1), 20.6−20.7 and 169.1−170.5 (7 acetates).

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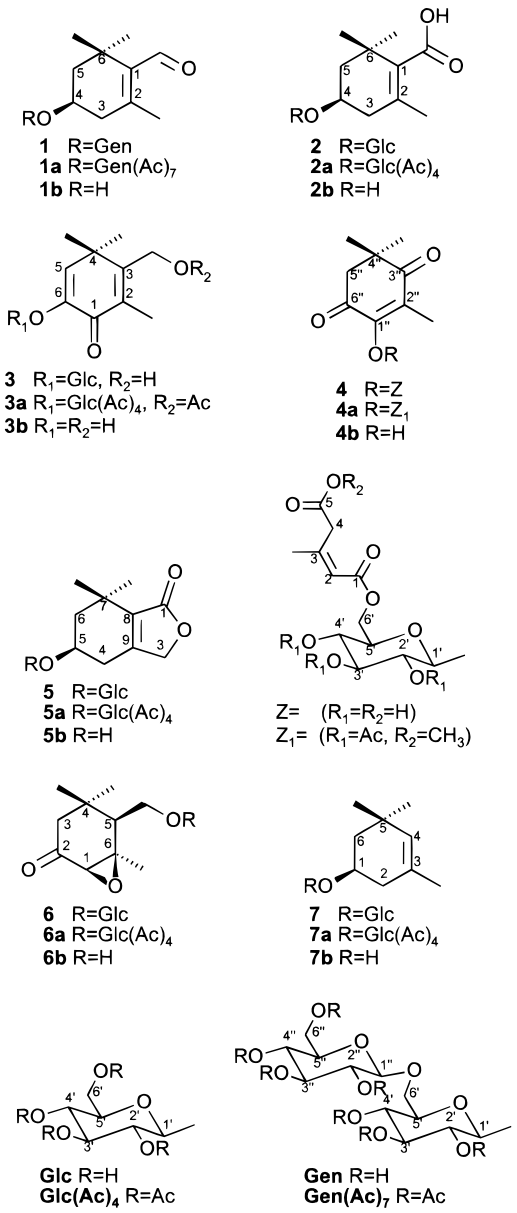

Figure 1. Structures of novel saffron constituents **1**−**7**.

*(4R)-4-Hydroxy-2,6,6-trimethylcyclohex-1-enecarboxylic acid O-β-*D*-glucopyranoside (**2**)* was isolated as tetraacetate (**2a**): 19.8 mg; $[\alpha]_D$ −14.6° (*c* 0.13% in MeOH); UV (MeOH) λ_{max} 260 nm; CD (*c* 0.003% in MeOH) $[\theta]_{206}$ +10054, $[\theta]_{234}$ −15829; IR (NaCl) ν 3437, 2960, 2924, 1753 (C═O), 1651 (C═O), 1368, 1226, 1176, 1065, 1039, 907, 756 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m*/*z* 532, indicating a molecular mass of 514 ($C_{24}H_{34}O_{12}$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.12 and 1.22 (2 × 3H, 2s, 2 Me-C6), 1.54 (1H, dd, J = 12.0, 12.0 Hz, H5$_a$), 1.75 (3H, s, Me-C2), 1.84 (1H, ddd, J = 12.0, 3.5, 1.0 Hz, H5$_b$), 2.01−2.08 (4 × 3H, 4s, 4 acetates), 2.10 (1H, dd, J = 16.5, 9.0 Hz, H3$_a$), 2.30 (1H, ddd, J = 16.5, 5.0, 1.0 Hz, H3$_b$), 3.72 (1H, ddd, J = 10.0, 5.0, 2.5 Hz, H5′), 4.02 (1H, dddd, J = 12.0, 9.0, 5.0, 3.5 Hz, H4), 4.13 (1H, dd, J = 12.5, 2.5 Hz, H6′$_a$), 4.25 (1H, dd, J = 12.5, 5.0 Hz, H6′$_b$), 4.63 (1H, d, J = 8.0 Hz, H1′), 4.96 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.06 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.22 (1H, dd, J = 9.5, 9.5 Hz, H3′); ^{1}H NMR (360 MHz, C_6D_6) δ 1.17 and 1.31 (2 × 3H, 2s, 2 Me-C6), 1.52 (1H, dd, J = 12.0, 12.0 Hz, H5$_a$), 1.68−1.78 (4 × 3H, 4s, 4 acetates), 1.71 (3H, s, Me-C5), 1.85 (1H, ddd, J = 12.0, 3.5, 1.0 Hz, H5$_b$), 1.89 (1H, ddd, J = 17.5, 9.0, 1.0 Hz, H3$_a$), 2.11 (1H, dd, J = 17.5, 5.5 Hz, H3$_b$), 3.31 (1H, ddd, J = 10.0, 5.0, 2.0 Hz, H5′), 3.93 (1H, dddd, J = 12.0, 9.0, 5.5, 3.5 Hz, H4), 4.08 (1H, dd, J = 12.5, 2.0 Hz, H6′$_a$), 4.23 (1H, dd, J = 12.5, 5.0 Hz, H6′$_b$), 4.35 (1H, d, J = 8.0 Hz, H1′), 5.24 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.30 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.45 (1H, dd, J = 9.5, 9.5 Hz, H3′); ^{13}C NMR (63 MHz, $CDCl_3$) δ 21.3 (Me-C2), 28.7 and 29.1 (2 Me-C6), 35.4 (C6), 37.6 (C3), 45.0 (C5), 62.3 (C6′), 68.6 (C4′), 71.5 (C2′), 71.8 (C5′), 72.8 (C3′), 73.0 (C4), 99.6 (C1′), 130.2 (C2), 135.9 (C1), 173.1 (COOH−C1), 20.6−20.7 and 169.4−170.6 (4 acetates); ^{13}C NMR (91 MHz, C_6D_6) δ 21.4 (Me-C2), 28.7 and 29.1 (2 Me-C6), 35.7 (C6), 38.2 (C3), 45.4 (C5), 62.0 (C6′), 68.9 (C4′), 72.1 (C2′), 72.2 (C5′), 72.2 (C4), 73.4 (C3′), 99.8 (C1′), 132.9 (C2), 134.8 (C1), 173.5 (COOH−C1), 20.1−20.3 and 168.7−170.1 (4 acetates). The tentative identification of **2** has been reported by Tarantilis et al. (1995).

*6-Hydroxy-3-(hydroxymethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one 6-O-β-*D*-glucopyranoside (**3**)* was isolated as pentaacetate (**3a**): 7.6 mg; $[\alpha]_D$ −21.8° (*c* 0.28% in MeOH); UV (MeOH) λ_{max} 250 nm; IR (NaCl) ν 3021, 2970, 2940, 1750 (C═O), 1653 (C═O), 1625 (C═C), 1434, 1368, 1225, 1121, 1038, 906, 756 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m*/*z* 572, indicating a molecular mass of 554 ($C_{26}H_{34}O_{13}$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.28 and 1.29 (2 × 3H, 2s, 2 Me-C4), 1.94 (3H, s, Me-C2), 2.02−2.12 (5 × 3H, 5s, 5 acetates), 3.74 (1H, ddd, J = 10.0, 5.0, 2.5 Hz, H5′), 4.15 (1H, dd, J = 12.5, 2.5 Hz, H6′$_a$), 4.22 (1H, dd, J = 12.5, 5.0 Hz, H6′$_b$), 4.82 (2H, s, H_2C−C3), 5.00 (1H, d, J = 8.0 Hz, H1′), 5.11 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.19 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.28 (1H, dd, J = 9.5, 9.5 Hz, H3′), 6.37 (1H, s, H5); ^{13}C NMR (63 MHz, $CDCl_3$) δ 11.4 (Me-C2), 25.8 and 25.9 (2 Me-C4), 39.8 (C4), 60.2 (OCH_2−C3), 62.1 (C6′), 68.5 (C4′), 71.1 (C2′), 72.0 (C5′), 72.5 (C3′), 99.0 (C1′), 136.5 (C2), 138.9 (C5), 146.1 (C6), 152.1 (C3), 180.8 (C1), 20.6−20.7 and 169.4−170.7 (5 acetates).

*(2Z)-3-Methylpent-2-enedioic acid 1-[1-(2,4,4-trimethyl-3,6-dioxo-cyclohexenyloxy)-β-*D*-glucopyranosid-6-yl] ester (**4**)* was isolated as peracetylated methylester (**4a**): 1.9 mg; $[\alpha]_D$ −20.0° (*c* 0.15% in MeOH); UV (MeOH) λ_{max} 213, 263 nm; IR (NaCl) ν 2961, 2932, 1758 (C═O), 1697 (C═O), 1676 (C═C), 1621, 1436, 1375, 1244, 1217, 1162, 1145, 1058, 1037, 996, 922, 907, 862, 759 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m*/*z* 614, indicating a molecular mass of 596 ($C_{28}H_{36}O_{14}$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.20 and 1.24 (2 × 3H, 2s, 2 Me-C4″), 1.91 (3H, s, Me-C2″), 1.98 (3H, d, J = 1.0 Hz, Me-C3), 2.02, 2.03 and 2.10 (3 × 3H, 3s, 3 acetates), 2.58 (1H, d, J = 15.5 Hz, H5″$_a$), 2.79 (1H, d, J = 15.5 Hz, H5″$_b$), 3.64 (1H, J = 16.0 Hz, H4$_a$), 3.70 (3H, s, Me-O), 3.72 (1H, ddd, J = 10.0, 4.5, 3.0 Hz, H5′), 3.75 (1H, d, J = 16.0 Hz, H4$_b$), 4.11 (1H, dd, J = 12.5, 4.5 Hz, H6′$_a$), 4.16 (1H, dd, J = 12.5, 3.0 Hz, H6′$_b$), 5.09 (1H, d, J = 10.0, 9.5 Hz, H4′), 5.17 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.26 (1H, dd, J = 9.5, 9.5 Hz, H3′), 5.68 (1H, d, J = 8.0 Hz, H1′), 5.81 (1H, q, J = 1.0 Hz, H2); ^{13}C NMR (91 MHz, $CDCl_3$) δ 10.1 (Me-C2″), 25.6 and 26.8 (2 Me-C4″), 25.8 (Me-C3), 38.3 (C4), 45.7 (C4″), 51.4 (C5″), 52.0 (Me-O), 61.2 (C6′), 68.4 (C4′), 71.5 (C2′), 72.2 (C5′), 72.6 (C3′), 97.6 (C1′), 118.2 (C2), 134.3 (C2″), 152.6 (C3), 154.4 (C1″), 165.2 (C1), 170.4 (C5), 193.1 (C6″), 202.2 (C3″), 20.6−20.7 and 169.4−170.1 (3 acetates).

*(5S)-5-Hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3H-isobenzofuran-1-one O-β-*D*-glucopyranoside (**5**)* was isolated as its tetraacetate (**5a**): 0.8 mg; $[\alpha]_D$ −26.7° (*c* 0.09% in MeOH); UV (MeOH) λ_{max} 231 nm; CD (*c* 0.002% in MeOH) $[\theta]_{213}$ −21939, $[\theta]_{302}$ −4669; IR (NaCl) ν 2953, 2918, 1749, 1668, 1493, 1367, 1225, 1170, 1040, 908, 756, 698 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m*/*z* 530, indicating a molecular mass of 512 ($C_{24}H_{32}O_{12}$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23 and 1.32 (2 × 3H, 2s, 2 Me-C7), 1.70 (1H, dd, J = 13.5, 11.0 Hz, H6$_a$), 1.97 (1H, ddd, J = 13.5, 3.5, 1.5 Hz, H6$_b$), 2.01−2.10 (4 × 3H, 4s, 4 acetates), 2.27 (1H, ddd, J = 18.0, 6.0, 1.5 Hz, H4$_a$), 2.61 (1H, dd, J = 18.0, 9.0 Hz, H4$_b$), 3.73 (1H, ddd, J = 10.0, 5.5, 2.5 Hz, H5′), 4.12 (1H, dddd, J = 11.0, 9.0, 6.0, 3.5 Hz, H5), 4.16 (1H, dd, J = 12.5, 2.5 Hz, H6′$_a$), 4.25 (1H, dd, J = 12.5, 5.5 Hz, H6′$_b$), 4.57 (1H, d, J = 17.0 Hz, H3$_a$), 4.66 (1H, d, J = 17.0 Hz, H3$_b$), 4.67 (1H, d, J = 8.0 Hz, H1′), 4.97 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.07 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.22 (1H, dd, J = 9.5, 9.5 Hz, H3′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.9 and 27.0 (2 Me-C7), 30.5 (C4), 31.9 (C7), 45.4 (C6), 62.2 (C6′), 68.5 (C4′), 70.4 (C3), 71.4 (C2′), 72.0 (C5′), 72.7 (C3′),

73.1 (C5), 99.7 (C1′), 133.2 (C8), 155.7 (C9), 169.4 (C1), 20.6−20.7 and 169.1−170.3 (4 acetates).

*(1R,5S,6R)-5-(Hydroxymethyl)-4,4,6-trimethyl-7-oxabicyclo-[4.1.0]heptan-2-one O-β-D-glucopyranoside (**6**)* was isolated as tetraacetate (**6a**): 14.2 mg; $[\alpha]_D$ −42.2° (*c* 1.03% in MeOH); UV (MeOH) λ_{max} 204, 252 nm; CD (*c* 0.03% in MeOH) $[\theta]_{210}$ −4335, $[\theta]_{232}$ −1032, $[\theta]_{303}$ −4186, $[\theta]_{388}$ +273; IR (NaCl) ν 3024, 2963, 2907, 1757 (C=O), 1666 (C=O), 1451, 1435, 1369, 1308, 1223, 1162, 1138, 1100 (−C−O−C−), 1039, 962, 910, 846, 806, 757 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m/z* 532, indicating a molecular mass of 514 ($C_{24}H_{34}O_{12}$); ^1H NMR (360 MHz, $CDCl_3$) δ 0.94 and 0.96 (2 × 3H, 2s, 2 Me-C4), 1.49 (3H, s, Me-C6), 1.68 (1H, d, J = 14.0 Hz, $H3_a$), 1.91 (1H, dd, J = 7.0, 4.0 Hz, H5), 2.01−2.10 (4 × 3H, 4s, 4 acetates), 2.61 (1H, d, J = 14.0 Hz, $H3_b$), 3.02 (1H, s, H1), 3.64 (1H, dd, J = 10.0, 7.0 Hz, H_aCH−C5), 3.73 (1H, ddd, J = 10.0, 5.0, 2.5 Hz, H5′), 4.18 (1H, dd, J = 12.5, 2.5 Hz, $H6'_a$), 4.22 (1H, dd, J = 10.0, 4.0 Hz, H_bCH−C5), 4.31 (1H, dd, J = 12.5, 5.0 Hz, $H6'_b$), 4.56 (1H, d, J = 8.0 Hz, H1′), 5.04 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.12 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.22 (1H, dd, J = 9.5, 9.5 Hz, H3′); ^{13}C NMR (63 MHz, $CDCl_3$) δ 23.9 (Me-C6), 27.1 and 28.6 (2 Me-C4), 38.2 (C4), 44.9 (C3), 47.3 (C5), 61.2 (C1), 62.0 (C6′), 66.4 (C6), 67.6 (OCH_2−C5), 68.5 (C4′), 71.1 (C2′), 71.9 (C5′), 72.8 (C3′), 100.7 (C1′), 206.7 (C2), 20.5−20.7 and 169.1−170.6 (4 acetates).

*(1R)-3,5,5-Trimethylcyclohex-3-enol O-β-D-glucopyranoside (**7**)* was isolated as tetraacetate (**7a**): 4.0 mg; $[\alpha]_D$ −4.0° (*c* 0.1% in MeOH); UV (MeOH) λ_{max} 206 nm; CD (*c* 0.003% in MeOH) $[\theta]_{207}$ −8326; IR (NaCl) ν 2957, 2926, 2860, 1756 (C=O), 1653 (C=C), 1435, 1368, 1224, 1039, 906, 881, 837 (>C=CH), 668 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m/z* 488, indicating a molecular mass of 470 ($C_{23}H_{34}O_{10}$); ^1H NMR (360 MHz, $CDCl_3$) δ 0.97 and 0.99 (2 × 3H, 2s, 2 Me-C5), 1.41 (1H, dd, J = 12.5, 12.0 Hz, $H6_a$), 1.64 (3H, d, J = 1.0 Hz, Me-C3), 1.80 (1H, ddd, J = 12.5, 4.0, 1.0 Hz, $H6_b$), 1.85 (1H, dd, J = 17.5, 9.5 Hz, $H2_a$), 2.01−2.08 (4 × 3H, 4s, 4 acetates), 2.16 (1H, ddd, J = 17.5, 6.0, 1.0 Hz, $H2_b$), 3.71 (1H, ddd, J = 10.0, 5.5, 3.0 Hz, H5′), 3.97 (1H, dddd, J = 12.0, 9.5, 6.0, 4.0 Hz, H1), 4.13 (1H, dd, J = 12.5, 3.0 Hz, $H6'_a$), 4.26 (1H, dd, J = 12.5, 5.5 Hz, $H6'_b$), 4.64 (1H, d, J = 8.0 Hz, H1′), 4.97 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.07 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.11 (1H, q, J = 1.0 Hz, H4), 5.22 (1H, dd, J = 9.5, 9.5 Hz, H3′); ^{13}C NMR (63 MHz, $CDCl_3$) δ 23.3 (Me-C3), 29.5 and 31.2 (2 Me-C5), 33.8 (C5), 36.3 (C2), 43.5 (C6), 62.3 (C6′), 68.7 (C4′), 71.6 (C2′), 71.8 (C5′), 72.9 (C3′), 74.6 (C1), 99.5 (C1′), 127.8 (C3), 132.1 (C4), 20.6−20.7 and 169.2−170.6 (4 acetates).

In addition to these novel natural products, the known glycosides **8**−**13** were also obtained in the pure form.

*(4R)-4-Hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carbaldehyde O-β-D-glucopyranoside (**8**)* was isolated as tetraacetate (**8a**): 70.3 mg; $[\alpha]_D$ −27.4° (*c* 0.71% in MeOH); UV (MeOH) λ_{max} 244 nm; IR ($CHCl_3$) ν 2956, 2872, 1757 (C=O), 1747 (C=O), 1672 (>C=C−CHO), 1615, 1458, 1432, 1376, 1335, 1230, 1176, 1125 (−C−O−C−), 1039, 908, 842, 757 cm^{-1}; CD (*c* 0.01% in MeOH) $[\theta]_{244}$ −15493, $[\theta]_{337}$ +2879; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m/z* 516, indicating a molecular mass of 498 ($C_{24}H_{34}O_{11}$); ^1H NMR (360 MHz, $CDCl_3$) δ 1.23 and 1.25 (2 × 3H, 2s, 2 Me-C6), 1.55 (1H, dd, J = 12.5, 12.0 Hz, $H5_a$), 1.85 (1H, ddd, J = 12.5, 3.0, 2.0 Hz, $H5_b$), 2.01−2.08 (4 × 3H, 4s, 4 acetates), 2.12 (3H, br s, Me-C2), 2.24 (1H, dd, J = 18.0, 9.0 Hz, $H3_a$), 2.48 (1H, ddd, J = 18.0, 5.5, 2.0 Hz, $H3_b$), 3.74 (1H, ddd, J = 10.0, 5.5, 2.5 Hz, H5′), 3.94 (1H, dddd, J = 12.0, 9.0, 5.5, 3.0 Hz, H4), 4.14 (1H, dd, J = 12.0, 2.5 Hz, $H6'_a$), 4.25 (1H, dd, J = 12.0, 5.5 Hz, $H6'_b$), 4.66 (1H, d, J = 8.0 Hz, H1′), 4.97 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.06 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.22 (1H, dd, J = 9.5, 9.5 Hz, H3′), 10.1 (1H, s, CHO−C1), ^{13}C NMR (63 MHz, $CDCl_3$) δ 19.0 (Me-C2), 27.6 and 28.6 (2 Me-C6), 35.4 (C6), 46.7 (C5), 41.1 (C3), 62.1 (C6′), 68.5 (C4′), 71.3 (C2′), 71.7 (C5′), 72.3 (C4), 72.7 (C3′), 99.6 (C1′), 140.1 (C1), 150.9 (C2), 191.3 (CHO−C1), 20.4−20.6 and 169.0−170.4 (4 acetates).

*(4S,3′R)-4-Hydroxy-4-(3′-hydroxy-1′-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one 3′-O-β-D-glucopyranoside (roseoside, **9**) as well as 2-phenylethyl O-β-D-glucopyranoside (**10**) and benzyl O-β-D-glucopyranoside (**11**)* were isolated as the respective tetraacetates (**9a**, 1.9 mg; **10a**, 2.0 mg; **11a**, 3.1 mg). For spectral data, cf. Otsuka et al. (1995) and Williams et al. (1983).

*(4S)-4-Hydroxydihydrofuran-2-one O-β-D-glucopyranoside (**12**)* was isolated as tetraacetate (**12a**): 4.8 mg; $[\alpha]_D$ +7.6° (*c* 0.21% in MeOH); UV (MeOH) λ_{max} 207 nm; CD (*c* 0.03% in MeOH) $[\theta]_{212}$ −9577; IR (NaCl) ν 3472, 3023, 2958 (C−H), 1748 (C=O), 1435, 1369, 1231, 1166, 1039, 911, 759 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m/z* 450, indicating a molecular mass of 432 ($C_{18}H_{24}O_{12}$); ^1H NMR (360 MHz, $CDCl_3$) δ 2.01−2.10 (4 × 3H, 4s, 4 acetates), 2.73 (1H, dd, J = 13.0, 3.0 Hz, $H3_a$), 2.75 (1H, dd, J = 13.0, 1.0 Hz, $H3_b$), 3.71 (1H, ddd, J = 10.0, 5.0, 2.5 Hz, H5′), 4.15 (1H, dd, J = 12.0, 2.5 Hz, $H6'_a$), 4.24 (1H, dd, J = 12.0, 5.0 Hz, $H6'_b$), 4.31 (1H, dd, J = 10.5, 2.0 Hz, $H5_a$), 4.39 (1H, dd, J = 10.5, 5.0 Hz, $H5_b$), 4.63 (1H, d, J = 8.0 Hz, H1′), 4.64 (1H, dddd, J = 5.0, 3.0, 2.0, 1.0 Hz, H4), 4.98 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.07 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.21 (1H, dd, J = 9.5, 9.5 Hz, H3′); ^{13}C NMR (91 MHz, $CDCl_3$) δ 20.5−20.7 (acetates), 35.6 (C3), 61.8 (C6′), 68.1 (C4′), 70.8 (C2′), 72.2 (C5′), 72.5 (C3′), 72.6 (C5), 74.0 (C4), 99.5 (C1′), 169.1−170.5 (acetates), 174.7 (C2).

*(4R)-4-Hydroxydihydrofuran-2-one O-β-D-glucopyranoside (**13**)* was isolated as tetraacetate (**13a**): 4.9 mg; $[\alpha]_D$ +17.6° (*c* 0.19% in MeOH); UV (MeOH) λ_{max} 207 nm; CD (*c* 0.03% in MeOH) $[\theta]_{212}$ −2430; IR (NaCl) ν 3482, 3023, 2960 (C−H), 1751 (C=O), 1435, 1370 (H_3C−COO), 1228, 1169, 1039, 910, 758 cm^{-1}; DCI-MS (reactant gas, NH_3) pseudo-molecular ion at *m/z* 450, indicating a molecular mass of 432 ($C_{18}H_{24}O_{12}$); ^1H NMR (360 MHz, $CDCl_3$) δ 2.01−2.10 (4 × 3H, 4s, 4 acetates), 2.71 (1H, dd, J = 13.0, 3.0 Hz, $H3_a$), 2.77 (1H, dd, J = 13.0, 1.0 Hz, $H3_b$), 3.71 (1H, ddd, J = 10.0, 4.5, 2.5 Hz, H5′), 4.16 (1H, dd, J = 12.5, 2.5 Hz, $H6'_a$), 4.23 (1H, dd, J = 12.5, 4.5 Hz, $H6'_b$), 4.25 (1H, dd, J = 10.5, 5.0 Hz, $H5_a$), 4.27 (1 H, dd, J = 10.5, 2.0 Hz, $H5_b$), 4.58 (1H, d, J = 8.0 Hz, H1′), 4.65 (1H, dddd, J = 5.0, 3.0, 2.0, 1.0 Hz, H4), 4.98 (1H, dd, J = 9.5, 8.0 Hz, H2′), 5.08 (1H, dd, J = 10.0, 9.5 Hz, H4′), 5.21 (1H, dd, J = 9.5, 9.5 Hz, H3′); ^{13}C NMR (91 MHz, $CDCl_3$) δ 20.4−20.6 (acetates), 34.9 (C3), 61.7 (C6′), 68.1 (C4′), 70.9 (C2′), 72.2 (C5′), 72.5 (C3′), 73.7 (C5), 74.1 (C4), 99.5 (C1′), 169.2−170.5 (acetates), 174.2 (C2).

Synthesis of (1*R*,5*R*,6*R*,3′*RS*)-5-(3′-Hydroxybutyl)-4,4,6-trimethyl-7-oxabicyclo[4.1.0]heptan-2-one (21). An authentic reference of blumenol *C*−*O*-*β*-D-glucopyranoside (Roscher and Winterhalter, 1993) was enzymatically hydrolyzed using sweet almond emulsin. Epoxidation of the liberated aglycon was performed with H_2O_2 in MeOH/NaOH (Klein and Ohloff, 1963). The so-obtained 1*R*,5*R*,6*R*-configured epoxide **21** was purified by flash chromatography. Spectral data of epoxide **21**: CD (*c* 0.05% in MeOH) $[\theta]_{211}$ −1499, $[\theta]_{299}$ −1287; $[\alpha]_D$ +13.5° (*c* 0.19% in MeOH); UV (MeOH) λ_{max} 202, 288 nm; IR (NaCl) ν 3413 (OH), 2964, 2929, 2873, 1710 (C=O), 1470, 1389, 1372, 1126, 1089 (−C−O−C−), 1028, 983, 957, 909, 888 cm^{-1}; R_i (DB-Wax) 2673; R_i (DB-5) 1714; EI-MS (70 eV), *m/z* (%) 226 (<1, $[M]^+$), 211 (<1, $[M - CH_3]^+$), 193 (1, $[M - H_2O - CH_3]^+$), 182 (2), 167 (1), 153 (6), 137 (5), 125 (14), 111 (32), 109 (17), 95 (22), 85 (11), 83 (64), 81 (21), 71 (15), 69 (19), 67 (22), 57 (16), 56 (23), 55 (44), 53 (18), 45 (45), 43 (100), 41 (61); ^1H NMR (360 MHz, $CDCl_3$) δ 0.83 and 1.09 (2 × 3H, 2s, 2 Me-C4), 1.25 (3H, d, J = 6.0 Hz, Me-C3′), 1.40 (1H, m, $H1'_a$), 1.47 (3H, br s, Me-C6), 1.55 (1H, m, $H2'_a$), 1.67 (1H, m, $H1'_b$), 1.70 (1H, m, $H2'_b$), 1.85 (1H, m, H5), 1.88 (1H, d, J = 15.0 Hz, $H3_a$), 2.61 (1H, d, J = 15.0 Hz, $H3_b$), 3.00 (1H, br s, H1), 3.83 (1H, m, H3′); ^{13}C NMR (63 MHz, $CDCl_3$) δ 22.1 (CH_3−C6), 22.9 and 30.2 (2 Me-C4), 23.7 (Me-C3′), 24.0 (C1′), 39.9 (C4), 40.8 (C2′), 49.7 (C5), 50.4 (C3), 62.7 (C1), 66.8 (C6), 68.2 (C3′), 207.7 (C2).

Aglycon and Sugar Analysis. After deacetylation with 0.02 M NaOMe in MeOH, each of the glycosides **1**−**7** in 1 mL of H_2O was acidified with 1 drop of diluted acetic acid and incubated overnight (37 °C) with 5 mg of *β*-glucosidase (sweet almond emulsin, Serva). The liberated aglycons were extracted with Et_2O (2 × 2 mL) and analyzed by GC/MS. The aqueous layer was passed through an ultrafilter (Ultrafree-MC 5000 NMGG, Millipore), and 20 μL of the enzyme-free filtrate was then injected into the HPLC system (Shandon Hypersil APS-5 μm column, 125 × 4.6 mm; eluent, acetonitrile/

H_2O 80:20). The presence of D-glucose was verified by on-line coupled refractive index (RI detector, Knauer, Berlin) and polarimetric detection (Chiralyzer polarimetric detector, IBZ Messtechnik, Hannover).

*(4R)-4-Hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carbaldehyde (**1b**/**8b**)*: sign of optical rotation, (−); R_i (DB-Wax) 2367; R_i (DB-5) 1543; EI-MS (70 eV), *m/z* (%) 168 (43, $[M]^+$), 153 (22, $[M - CH_3]^+$), 150 (24, $[M - H_2O]^+$), 139 (10), 135 (100, $[M - H_2O - CH_3]^+$), 122 (18), 121 (53), 117 (10), 109 (27), 108 (14), 107 (80), 106 (7), 105 (27), 95 (15), 93 (21), 91 (55), 81 (30), 79 (54), 77 (25), 69 (24), 67 (19), 65 (12), 55 (48), 53 (23), 51 (11), 43 (49), 41 (48).

*(4R)-4-Hydroxy-2,6,6-trimethyl-1-cyclohexenecarboxylic acid (**2b**)*: sign of optical rotation, (−); R_i (DB-Wax) >2800; R_i (DB-5) 1530; EI-MS (70 eV), *m/z* (%) 184 (3, $[M]^+$), 170 (3), 169 (37, $[M - CH_3]^+$), 167 (5), 166 (37, $[M - H_2O]^+$), 152 (10), 151 (100, $[M - H_2O - CH_3]^+$), 139 (17), 125 (20), 123 (15), 121 (41), 107 (54), 105 (35), 95 (30), 93 (13), 91 (47), 83 (18), 81 (13), 79 (38), 77 (26), 69 (15), 67 (23), 65 (10), 59 (18), 55 (31), 53 (22), 51 (10), 43 (60), 41 (44).

*6-Hydroxy-3-(hydroxymethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one (**3b**)*: R_i (DB-Wax) 2754; R_i (DB-5) 1555; EI-MS (70 eV), *m/z* (%) 182 (17, $[M]^+$), 167 (5, $[M - CH_3]^+$), 164 (19, $[M - H_2O]^+$), 154 (11), 152 (39), 151 (29), 149 (18, $[M - H_2O - CH_3]^+$), 139 (20), 138 (63), 137 (54), 136 (46), 135 (20), 125 (12), 124 (33), 123 (41), 122 (52), 121 (100), 109 (23), 108 (17), 107 (30), 105 (10), 95 (15), 93 (49), 91 (45), 83 (12), 81 (10), 79 (28), 78 (12), 77 (55), 69 (14), 67 (25), 65 (19), 55 (36), 53 (31), 51 (18), 43 (55), 41 (36).

*2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1,4-dione (**4b**)*: R_i (DB-Wax) 2080; R_i (DB-5) 1236; EI-MS (70 eV), *m/z* (%) 168 (36, $[M]^+$), 153 (28, $[M - CH_3]^+$), 150 (1, $[M - H_2O]^+$), 140 (26), 126 (50), 125 (27), 85 (27), 84 (100), 83 (43), 69 (42), 57 (37), 56 (77), 55 (41), 53 (11), 43 (20), 41 (35).

*(5S)-5-Hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3H-isobenzofuran-1-one (**5b**)*: sign of optical rotation, (−); R_i (DB-Wax) 2529; R_i (DB-5) 1677; EI-MS (70 eV), *m/z* (%), 182 (10, $[M]^+$), 167 (25, $[M - CH_3]^+$), 164 (14, $[M - H_2O]^+$), 163 (10), 150 (10), 149 (100, $[M - H_2O - CH_3]^+$), 139 (27), 138 (23), 136 (27), 121 (35), 119 (16), 109 (22), 105 (19), 95 (15), 93 (57), 91 (37), 81 (22), 79 (33), 77 (39), 67 (19), 65 (16), 55 (30), 53 (22), 51 (15), 43 (47), 41 (41).

*(1R,5S,6R)-5-(Hydroxymethyl)-4,4,6-trimethyl-7-oxabicyclo-[4.1.0]heptan-2-one (**6b**)*: sign of optical rotation, (−); R_i (DB-Wax) 2333; R_i (DB-5) 1397; EI-MS (70 eV), *m/z* (%) 184 (<1, $[M]^+$), 169 (8, $[M - CH_3]^+$), 166 (2, $[M - H_2O]^+$), 111 (10), 98 (38), 97 (12), 87 (30), 86 (13), 85 (16), 84 (10), 83 (100), 71 (16), 69 (16), 67 (11), 56 (18), 55 (33), 53 (13), 43 (53), 41 (38).

*(1R)-3,5,5-Trimethyl-3-cyclohexen-1-ol (**7b**)*: sign of optical rotation, (−); R_i (DB-Wax) 1605; R_i (DB-5) 1059; EI-MS (70 eV), *m/z* (%) 140 (14, $[M]^+$), 125 (25, $[M - CH_3]^+$), 122 (15, $[M - H_2O]^+$), 107 (100, $[M - H_2O - CH_3]^+$), 96 (15), 95 (16), 91 (15), 81 (21), 79 (15), 55 (19), 43 (10), 41 (13).

*4-Hydroxydihydrofuran-2-one (isomers **12b** and **13b**)*: sign of optical rotation, **12b** (−); **13b** (+); R_i (DB-Wax) 1739; R_i (DB-5) 923; EI-MS (70 eV), *m/z* (%) 102 (<1, $[M]^+$), 84 (46, $[M - H_2O]^+$), 55 (100), 54 (20), 53 (3).

High-Resolution Gas Chromatography (HRGC). Dani educational gas chromatographs equipped with either a J&W fused silica DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) or 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 (1:20) was employed. The temperature program was as follows: 60 °C (2 min) to 300 °C at 5 °C/min for DB-5 and from 50 °C (3 min) to 215 °C at 4 °C/min for DB-Wax, respectively. The flow rate for the carrier gas was 1.5 mL/min of He, for the makeup gas, 30 mL/min of N_2, and for the detector gases, 37 mL/min of H_2 and 280 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 250 °C, respectively. The linear retention index (R_i) is based on a series of *n*-hydrocarbons.

HRGC/Mass Spectrometry (HRGC/MS). HRGC/MS was performed with a Hewlett-Packard GCD system equipped with a PTV injector (KAS system, Gerstel, Mühlheim, Germany). 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, 1.2 mL/min of He; temperature of ion source, 180 °C; electron energy, 70 eV; injection volumes, 1 μL.

Nuclear Magnetic Resonance (NMR). ^1H and ^{13}C NMR spectral data were recorded on Fourier transform Bruker AM 360 and AC 250 as well as JEOL Lambda-500 spectrometers with TMS as internal reference standard. Signals were assigned by $^1H-^1H$ COSY and $^1H-^{13}C$ COSY, as well as HMBC experiments.

Desorption Chemical Ionization Mass Spectrometry (DCI-MS). DCI-MS was carried out with a Finnigan TSQ 70 mass spectrometer at 70 eV using ammonia as reactant gas and ion source temperature and pressure of 150 °C and 1.5×10^{-4} mbar, respectively, as well as a temperature gradient of 400 °C/min. Mass range was 60−900.

Circular Dichroism (CD). CD spectra were recorded in MeOH (20 °C) using a Jasco J-710 polarimeter.

Infrared Spectroscopy (IR). IR spectra were recorded in KBr and $CHCl_3$ or on NaCl with a Jasco FT/IR-410 Fourier transform infrared spectrometer.

Ultraviolet−Visible Absorption Spectroscopy. UV spectra were recorded on a Jasco V-530 UV−vis spectrophotometer.

Optical Rotation. $[\alpha]_D$ values were measured on a Perkin-Elmer 241 polarimeter.

RESULTS AND DISCUSSION

Safranal (2,6,6-trimethylcyclohexa-1,3-dienecarbaldehyde, **14**) is the principal aroma compound of saffron. Its generation through cleavage of the nonvolatile precursor picrocrocin (**8**) is well established (Alonso et al., 1996). In addition to safranal, higher oxygenated constituents with a C_9- or C_{10}- carbon skeleton have been identified as early as 1971 by Zarghami and Heinz. More recently, Rödel and Petrizka (1991) as well as Tarantilis and Polisiou (1997) identified further carotenoid-derived aroma volatiles in saffron by using GC/MS analysis. It is noteworthy that the structures of several of these compounds still have to be considered as tentative, since the identifications are based on the interpretation of the MS data alone. Moreover, for most of the detected volatiles, pathways of formation remain to be elucidated.

In a recent paper (Straubinger et al., 1997), we reported the identification of novel carotenoid metabolites in saffron, which included the β-D-glucopyranosides of (4*R*)-4-hydroxy-3,5,5-trimethylcyclohex-2-enone (**15**), (4*S*)-4-hydroxy-3,5,5-trimethylcyclohex-2-enone (**16**), and (4*S*)-4-(hydroxymethyl)-3,5,5-trimethylcyclohex-2-enone (**17**). After further purification of the remaining subfractions of the MeOH isolate, seven additional carotenoid metabolites (**1**−**7**) could be obtained in the pure form. Their structures were unambiguously deduced using one- and two-dimensional NMR methods. Assignment of the stereochemistry in compounds **1**, **2**, and **5**−**7** was made by CD spectroscopy using published CD data of structurally related compounds [cf. Buchecker and Eugster (1973), Galbraith and Horn (1973), Mayer and Santer (1980), Miyase et al. (1988), Ohloff et al. (1973), and Uebelhardt et al. (1986)]. In the case of epoxide **6**, the absolute configuration was clarified by comparing its CD data with those of authentic (1*R*,5*R*,6*R*,3′*RS*)-5-(3′-hydroxybutyl)-4,4,6-trimethyl-7-oxabicyclo[4.1.0]heptan-2-one (**21**).

Compounds **1**−**6** are—to the best of our knowledge—reported here for the first time. Glucoside **7** has earlier been synthesized by Skouroumounis et al. (1995). In addition to the novel natural compounds **1**−**7**, five

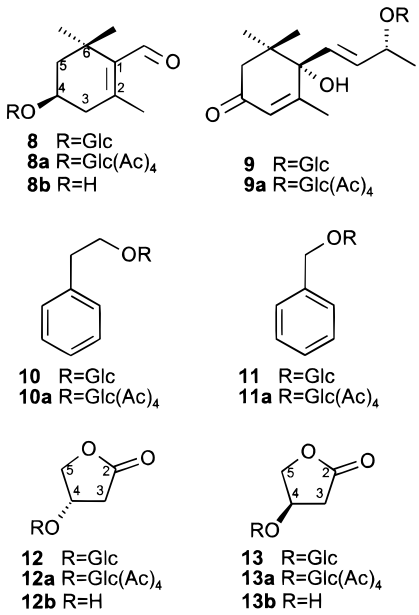

Figure 2. Structures of additional glycoconjugates **8**−**13** isolated from saffron.

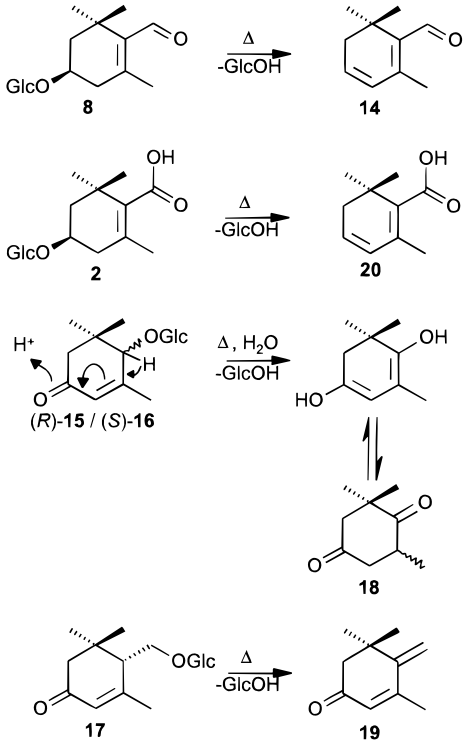

Figure 3. Formation of saffron volatiles **14**, **18**, **19**, and **20** from the respective nonvolatile precursors **8**, **15**/**16**, **17**, and **2**.

known glucosides (**8**−**12**) and a novel stereoisomer of glucoside **13** (Ainslie et al., 1986; Ito et al., 1993), that is, (4*S*)-4-hydroxydihydrofuran-2-one O-β-D-glucopyranoside (**12**), could be isolated from the methanolic extract of saffron (cf. Figure 2).

Precursor Function of Compounds 1−7 and 15−17. In their recent publication on volatile constituents of saffron, Tarantilis and Polissiou (1997) identified inter alia compounds **18**−**20** by using GC/MS analyses. Several of these volatiles were presumed to be degradation products of saffron carotenoids resulting from the action of heat and oxygen on the ingredients (Kanasawud and Crouzet, 1990a,b). Acid **20** was discussed as a likely oxidation product, chemically formed from safranal **14**. The detection of glycoconjugates **1**−**7** and **15**−**17** reveals that a number of the newly detected saffron compounds are most likely degradation products which are formed under the workup conditions from nonvolatile aroma progenitors. In the case of glycoconjugates **2** and **15**−**17**, hydrolytic cleavage of the glycoconjugates in combination with dehydration reactions rationalizes the formation of aroma volatiles **14** and **18**−**20** under the vigorous isolation conditions of steam distillation (cf. Figure 3).

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LITERATURE CITED

Ainslie, R. D.; Moore, R. E.; Patterson, G. M. (*S*)−(−)-3,4-Dihydroxybutanoic acid γ-lactone from Puerto Rican *Lyngbya majuscula*. *Phytochemistry* **1986**, *25*, 2654−2655.

Alonso, G. L.; Salinas, M. R.; Esteban-Infantes, F. J.; Sánchez-Fernández, M. A. Determination of safranal from Saffron (*Crocus sativus* L.) by Thermal Desorption-Gas Chromatography. *J. Agric. Food Chem.* **1996**, *44*, 185−188.

Buchecker, R.; Eugster, C. H. Absolute configuration of picrocrocin. *Helv. Chim. Acta* **1973**, *56*, 1121−1124.

Galbraith, M. N.; Horn, D. H. Stereochemistry of the blumenols: Conversion of blumenol A into (*S*)−(+)-abscisic acid. *J. Chem. Soc., Chem. Commun.* **1973**, 566−567.

Ito, A.; Kasai, R.; Yamasaki, K.; Sugimoto, H. Aliphatic and aromatic glucosides from *Anoectochilus koshunensis*. *Phytochemistry* **1993**, *33*, 1133−1137.

Kanasawud, P.; Crouzet, J. C. Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 1. β-Carotene degradation. *J. Agric. Food Chem.* **1990a**, *38*, 237−243.

Kanasawud, P.; Crouzet, J. C. Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueus medium. 2. Lycopene degradation. *J. Agric. Food Chem.* **1990b**, *38*, 1238−1242.

Klein, E.; Ohloff, G. Steric course of the alcaline epoxidation of α,β-unsaturated carbonyl compounds of cyclic monoterpenes. *Tetrahedron* **1963**, *19*, 1091−1099.

Mayer, H.; Santer J. M. Synthesis of optical active natural carotenoids and structural related natural products. VI. Synthesis of picrocrocin. *Helv. Chim. Acta* **1980**, *63*, 1463−1466.

Miyase, T.; Ueno, A.; Takizawa, N.; Kobayashi, H.; Oguchi, H. Studies on the glycosides of *Epimedium grandiflorum* Morr. var. *thunbergianum* (Miq.) Nakai. III. *Chem. Pharm. Bull.* **1988**, *36*, 2475−2484.

Ohloff, G.; Otto, E.; Rautenstrauch, V.; Snatzke, G. Circulardichroism; Chiroptical properties of some trimethylcyclohexen derivatives: The ionones, irones and abscisinic acids. *Helv. Chim. Acta* **1973**, *56*, 1874−1882.

Otsuka, H.; Yao, M.; Kamada, K.; Takeda, Y. Alangionosides G−M: Glycosides of megastigmane derivatives from the leaves of *Alangium prenifolium*. *Chem. Pharm. Bull.* **1995**, *43*, 754−759.

Rödel, W.; Petrzika, M. Analysis of the volatile components of saffron. *J. High Resolut. Chromatogr.* **1991**, *14*, 771−774.

Roscher, R.; Winterhalter, P. Application of multilayer coil countercurrent chromatography for the study of Vitis vinifera cv. Riesling leaf glycosides. *J. Agric. Food Chem.* **1993**, *41*, 1452−1457.

Skouroumounis, G. K.; Massy-Westropp, R. A.; Sefton, M. A.; Williams, P. J. Synthesis of glucosides related to grape and wine aroma precursors. *J. Agric. Food Chem.* **1995**, *43*, 974−980.

Straubinger, M.; Jezussek, M.; Waibel, R.; Winterhalter, P. Novel glycosidic constituents from Saffron. *J. Agric. Food Chem.* **1997**, *45*, 1678−1681.

Tarantilis, P. A.; Polissiou, M. G. Isolation and identification of the aroma components from saffron (*Crocus sativus*). *J. Agric. Food Chem.* **1997**, *45*, 459−462.

Tarantilis, P. A.; Tsoupras, G.; Polissiou, M. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV−visible photodiode-array detection-mass spectrometry. *J. Chromatogr. A* **1995**, *699*, 107−118.

Uebelhardt, P.; Baumeler, A.; Haag, A.; Prewo, R.; Bieri, J. H.; Eugster, C. H. Optically active 4,5-epoxy-4,5-dihydro-α-ionones; Synthesis of the stereoisomeric 4,5:4′,5′-diepoxy-4,5,4′,5′-tetrahydro-ϵ,ϵ-carotenes and the steric course of their hydrolysis. *Helv. Chim. Acta* **1986**, *69*, 816−834.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. Glycosides of 2-phenylethanol and benzyl alcohol in *Vitis vinifera* grapes. *Phytochemistry* **1983**, *22*, 2039−2041.

Zarghami, N. S.; Heinz, D. E. Monoterpene aldehydes and isophorone-related compounds of saffron. *Phytochemistry* **1971**, *10*, 2755−2761.

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