

Research Note

Isolation of 2-ethyl-3-methylmaleimide N- β -D-glucopyranoside from Riesling wine

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S u m m a r y : A glycosidic isolate of Riesling wine was separated with multilayer coil countercurrent chromatography (MLCCC). After acetylation and subsequent purification by high performance liquid chromatography (HPLC), the N- β -D-glucopyranoside of 2-ethyl-3-methylmaleimide (3-ethyl-4-methyl-1H-pyrrole-2,5-dione) **2** was identified for the first time as natural wine constituent. The identification was carried out by mass spectrometry (EI-MS, DCI-MS) as well as nuclear magnetic resonance spectroscopy (^1H NMR, ^{13}C NMR, COSY, HMBC).

K e y w o r d s : 2-ethyl-3-methylmaleimide N- β -D-glucopyranoside, aroma precursor, chlorophyll degradation, multilayer coil countercurrent chromatography, wine.

Introduction: Differences observed in the composition of acid and enzymatic hydrolysates of grape juices and wine have led to speculations about the presence of glycoconjugates that may be in part or fully resistant to enzymatic cleavage reactions (WILLIAMS *et al.* 1989; WILLIAMS 1993). A striking example is the substituted maleimide **1**. This aroma compound of tobacco (DEMOLE and BERTHET 1972, FUJIMORI *et al.* 1976) and tea (KAWAKAMI *et al.* 1995, SHIMODA *et al.* 1995) was recently also identified in grape juices (SEFTON *et al.* 1993, 1996). Substantial amounts of **1** could only be detected in the acid-released fractions of Chardonnay (SEFTON *et al.* 1993) and Semillon juices (SEFTON *et al.* 1996), whereas in the enzymatic hydrolysates only trace amounts of **1** were present. During our continuing studies on non-volatile compounds of Riesling wine (WINTERHALTER *et al.* 1997), we were able to identify a glycosidically bound form of 2-ethyl-3-methylmaleimide **1**. The isolation and structural elucidation of this novel wine constituent is reported in the present paper.

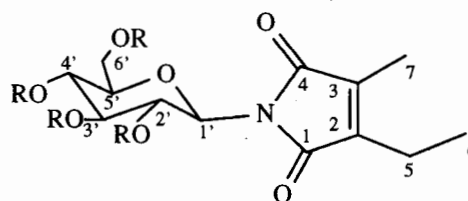
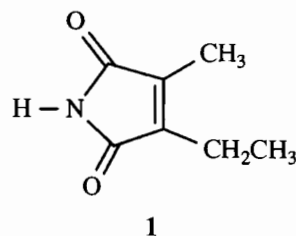
Material and methods: A commercial Riesling wine (100 l, QbA quality, Rheinpfalz, 1992 vintage) was dealcoholized prior to work-up.

The dealcoholized wine was passed through a column of Amberlite XAD-2 resin (GÜNATA *et al.* 1985). After rinsing with H_2O , the retained material was eluted with MeOH. The methanolic eluate was concentrated under reduced pressure and the remaining volatiles were removed by Et_2O extraction. For the initial fractionation of the isolate (20 g), multilayer coil countercurrent chromatography (MLCCC) was used (Multilayer Coil Separator-Extractor, P.C. Inc., Potomac, USA; equipped with a 85 m x 2.6 mm i.d. PTFE tubing;

solvent system: $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 7:13:8). MLCCC separated 7 fractions of which MLCCC fraction IV was rechromatographed using the analytical MLCCC device (160 m x 1.6 mm i. d. PTFE tubing; solvent system: $\text{EtOAc}/\text{BuOH}/\text{H}_2\text{O}$ 3:2:5). The obtained subfraction IV.2 was acetylated (Ac_2O in pyridine) and after flash chromatography (STILL *et al.* 1978) finally purified by preparative HPLC (Europher RP18 column, 5 μm , 250 mm x 16 mm, Knauer Säulentechnik, Berlin; eluent: $\text{MeOH}/\text{H}_2\text{O}$ 8:2).

The following instrumentation was used: NMR: Bruker AM 360 (CDCl_3 , chemical shifts in ppm); MS: Finnigan TSQ 70. Spectral data of the peracetylated N-glucoside of 2-ethyl-3-methylmaleimide (**2a**): 2.5 mg. EI-MS (70 eV): m/z 289 (52), 247 (52), 234 (75), 168 (100), 141 (39), 140 (44), 97 (42), 85 (37), 71 (53), 57 (52), 43 (85). DCI-MS: pseudo-molecular ion at m/z 487 [$\text{M}(469) + \text{NH}_4^+$], $\text{C}_{21}\text{H}_{27}\text{O}_{11}\text{N}$. ^1H -NMR (360 MHz): δ 1.14 (3H, t, $J = 7.5$ Hz, H_3 -6), 1.98 (3H, s, H_3 -7), 1.93 - 2.08 (4 x 3H, 4s, acetates), 2.42 (2H, br q, $J = 7.5$ Hz, H_2 -5), 3.79 (1H, ddd, $J = 9.5, 4.5, 2.5$ Hz, H_5 -1'), 4.15 (1H, dd, $J = 12.5, 2.5$ Hz, H_a -6'), 4.20 (1H, dd, $J = 12.5, 4.5$ Hz, H_b -6'), 5.21 (1H, d, $J = 9.5$ Hz, H_1 -1'), 5.22 (1H, t; $J = 9.5$ Hz, H_4 -4'), 5.27 (1H, t, $J = 9.5$ Hz, H_3 -3'), 5.92 (1H, t, $J = 9.5$ Hz, H_2 -2'). ^{13}C -NMR (90 MHz): δ 8.6 (C-7), 12.4 (C-6), 17.2 (C-5), 20.4 - 20.7 (acetates), 61.8 (C-6'), 67.9 (C-4'), 68.1 (C-2'), 73.7 (C-3'), 74.5 (C-5'), 77.6 (C-1'), 137.5 (C-3), 143.0 (C-2), 169.4 (C-1), 170.2 (C-4), 169.5 - 170.7 (acetates).

Results and Discussion: The glycosidic XAD-2 isolate from Riesling wine was fractionated by means of preparative as well as analytical MLCCC (ITO 1986; ROSCHER and WINTERHALTER 1993). After acetylation the subfractions were further purified by flash chromatography and RP-HPLC. The ^1H -NMR spectral data of purified compound **2a** contained all the signals of a peracetylated β -glucopyranosyl moiety. Whereas the magnitude of the vicinal coupling constants were coincident with data published for other β -gluco-pyranosides (ROSCHER and WINTERHALTER 1993), distinct downfield shifts for the protons at C1' and C2' were observed. Moreover, DCI-MS analyses revealed an odd molecular mass (469 amu). Thus,



2 R = H
2a R = Ac

the presence of a nitrogen-containing aglycone was evident. In addition to the sugar proton resonances, only 3 additional signals were apparent for the aglycone moiety, i.e. an ethyl group (two-proton quartet at δ 2.42 and three-proton triplet at δ 1.14) as well as a methyl group (three-proton singlet at δ 1.98). The ^{13}C -NMR spectrum showed 4 additional quaternary carbon atoms, i.e. two olefinic carbons (δ 137.5 and 143.0) and two carbonyl carbons (δ 169.4 and 170.2). Their connectivity was finally elucidated using a heteronuclear multiple-bond correlation (HMBC) experiment. On the basis of the NMR spectral data, the isolated compound was identified as peracetylated N- β -D-glucopyranoside of 2-ethyl-3-methylmaleimide **2a**. This N-glucoside has recently been independently identified as a constituent of mangosteen (*Garcinia mangostana*) leaves (KRAJEWSKI *et al.* 1996).

While oxygenated cleavage products of carotenoids are well-known grape and wine constituents (SEFTON *et al.* 1993, WINTERHALTER 1994), little is known about breakdown products of other plant pigments. Two apparently chlorophyll-derived compounds are the above mentioned 2-ethyl-3-methylmaleimide and its 2-vinyl analogue, which have been identified in the acid hydrolysates of Chardonnay and Semillon juices (SEFTON *et al.* 1993, 1996). The formation of 2-ethyl-3-methylmaleimide through oxidative degradation of chlorophyll has been reported for the first time by ELLSWORTH and ARONOFF (1968), and since then confirmed by other groups (JEN and MACKINNEY 1970, RONTANI *et al.* 1991).

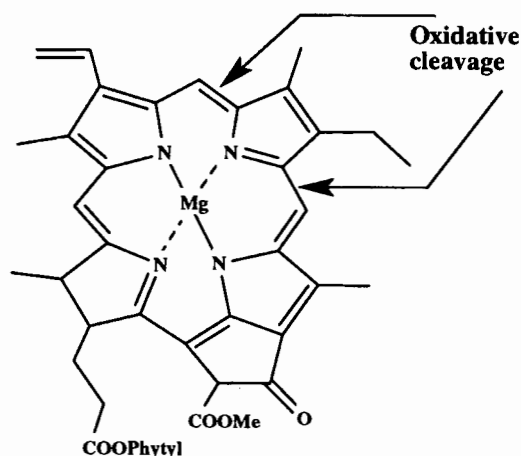


Figure: Hypothetic oxidative cleavage of chlorophyll giving rise to a formation of aglycone **1**.

With regard to the aroma precursor function of glycoside **2**, the so far published data are controversial. Whereas in the case of grape juices, acid hydrolyses led to a liberation of 2-ethyl-3-methylmaleimide **1**, presumably from the bound form **2**, experiments carried out by KRAJEWSKI *et al.* (1996) suggest that the N-glucoside **2** is largely resistant to acid hydrolysis. This obvious discrepancy has to be addressed in future studies.

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