

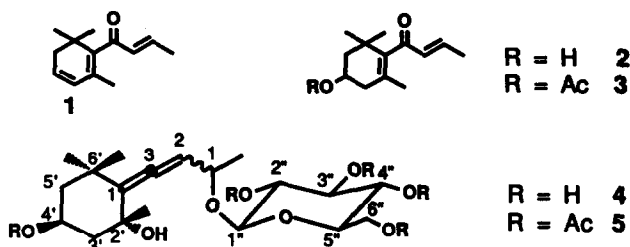
ISOLATION OF A GLUCOSIDIC PRECURSOR OF DAMASCENONE
FROM *LYCIUM HALIMIFOLIUM* MIL.

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Summary:

A precursor of damascenone (1), 3-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene)-1-methylprop-2-enyl β -D-glucopyranoside (4), was isolated from the leaves of *Lycium halimifolium* Mil. (Solanaceae) and characterized as its pentaacetate 5.

The essential oil of the leaves of *Lycium halimifolium* Mil. (Solanaceae) contains damascenone (1) as the main product [1]. This plant was therefore a convenient starting material for the isolation of a possible precursor of the norisoprenoid ketone 1.



Frozen leaves and fine stems (4.2 kg) of *Lycium halimifolium* Mil., picked near Saxon in Valais (Switzerland), were suspended in water (1.5 l), reduced to a slurry with a mixer, and covered with methanol (8 l). After 1 h, the suspension was filtered and the residue again taken up in methanol (2.5 l) overnight. The combined methanol extracts were concentrated to dryness. The solid residue (224.7 g) was dissolved in water (500 ml) and extracted with ether (removal of the chlorophylls). The water layer was treated with 1-butanol (2 l). - From now on, the presence of the precursor of 1 was checked for every separation step by an acid hydrolysis experiment: in a *Likens-Nickerson* apparatus, some mg of the fraction in a phosphate buffer (pH 3.0) were co-distilled with pentane. 1 was identified by GC/MS (Finnigan 4021C, Supelcowax 60 m, 0.25 mm id.). - After addition of toluene (1000ml) to the butanol extract and evaporation of the solvents, a dry extract (11 g) was obtained, which was stirred for 24 h with acetic anhydride (110 ml) and pyridine (110 ml). Azeotropic evaporation of the solvents after addition of toluene (450 ml) gave a mixture of peracetylated glycosides (12 g). These were chromatographed 4 times on silica gel (0.040- 0.063 mm) eluting with hexane/ EtOAc = 3/2, hexane/ EtOAc = 1/1, CHCl_3 and

toluene/ EtOAc = 1/1 respectively. At this stage, the thin layer plate, developed with anisaldehyde/ H₂SO₄, showed one pink spot (R_f = 0.65, toluene/ EtOAc = 3/2, 0.8 mg). The desorption chemical ionisation experiment using ammonia as reagent gas (VG 70 SE-250 mass spectrometer) indicated M⁺ = 598 (616 - 18, molecular formula C₂₉H₄₂O₁₃) and the fragments 538 (M⁺ - acetic acid), 331 (glucose tetraacetate), 268 (aglycone) and 250 (aglycone -H₂O). ¹H-NMR-spectrum (Bruker WH 360, CDCl₃, ppm, J in Hz): 1.08 (s, 3H, C(6')-CH₃ eq.); 1.25 (d, J = 6, 3H, C(1)-CH₃); 1.26 (s, 3H, C(2')-CH₃, eq.); 1.35 (s, 3H, C(6')-CH₃ ax.); 1.44 (dxd, J₁ = 13, J₂ = 13, 1H, C(5') or C(3') ax.); 1.96 (d, J = 13, 1H, C(3') or C(5') eq.); 2.0-2.15 (5s, 15H, acetates); ~ 2.10 (m, 1H, C(3') or C(5') ax., presence confirmed by decoupling); 2.25 (d, J = 13, 1H, C(3') or C(5') eq.); 3.65 (m, 1H, C(5'')); 4.12 (m, 1H, C(6'')); 4.26 (m, 1H, C(6'')); 4.29 (dxq, J₁ = 6, J₂ = 6, 1H, C(1)); 4.60 (d, J = 8, 1H, C(1'')); 4.96 (dxd, J₁ = 8, J₂ = 9, 1H, C(2'')); 5.07 (dxd, J₁ = 8, J₂ = 8, 1H, C(4'')); 5.19 (dxd, J₁ = 8, J₂ = 8, 1H, C(3'')); 5.35 (m, 1H, C(4'')); 5.40 (d, J = 6, 1H, C(2)). All these spectral data are in good agreement with 3-(4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexylidene)-1-methylprop-2-enyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (5). NOE-measurements allowed the determination of the stereochemistry C(4') and at C(2') : irradiation a) at 1.08 ppm increased the signals at 4.60 and 5.40 ppm, at b) 1.25 and 1.26 ppm increased the signals at 4.29, 4.60 and 5.40 ppm and at c) 1.35 ppm increased the signal at 5.35 ppm. (for ¹H-NMR data of carotenoides with similar substructures see [2] and [3]). Methanolysis (MeOH/ 0.2M NaOMe, 30 min, rt.) followed by enzymatic cleavage with β-glucosidase (EC 3.2.1.21, *Fluka*) in a phosphate buffer at pH 5.0 gave damascenone (1) and 3-hydroxy-β-damascone (2). By acidic hydrolysis (phosphate buffer, pH 3.0, *Likens-Nickerson*, pentane) 1 and 3-acetoxy-β-damascone (3) were obtained. Spectral data of 3: MS (70eV, m/e(%)): M⁺ = 250(0.5), 235(0.5), 190(15), 175(10), 121(77), 69(100), 61(2), 43(30), 41(26); ¹H-NMR (360MHz, CDCl₃, J in Hz): 1.01 (s, 3H); 1.18 (s, 3H); 1.54 (s, 3H); 1.92 (d, J = 7, 3H); 2.05 (s, 3H); 5.12 (m, 1H); 6.16 (d, J = 15, 1H); 6.72 (dxd, J₁ = 15, J₂ = 7, 1H). The isolation of the glucoside of the allenic triol 4 supports the hypothesis of Ohloff et al. [4], Isoe et al. [5] and recently by Sefton et al. [6], that damascenone (1) is derived from neoxanthin.

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