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VIOLAXANTHIN ESTERS FROM *VIOLA TRICOLOR* FLOWERS

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Key Word Index—*Viola tricolor*; Violaceae; violaxanthin ester; β-hydroxy acids.

Abstract The pattern of fatty acid esters of violaxanthin and the minor xanthophylls in the petals of *Viola tricolor* (yellow varieties) is unusually complex. This is due to the fact that β-hydroxy acids (12:0, 14:0, 16:0) take part in the esterification in addition to the usual acids (12:0, 14:0, 16:0, 18:0).

The xanthophylls in flower petals are usually esterified with saturated C_{12}–C_{18} acids ([1–3]; for review see [4]). The yellow varieties of *Viola tricolor* contain as their main pigment violaxanthin (*ca* 75% of total carotenoid) which occurs in an unusually complex ester pattern as has been shown by TLC [5]. In the present investigation the acid moieties of these violaxanthin esters have been analysed.

The source of carotenoid esters were whole petals or isolated lipid globules (plastoglobuli) in which the esters are mainly localized [6]. Violaxanthin diesters were the main components while monoesters and free violaxanthin only occurred in trace amounts. The diesters were separated by Si gel TLC into three fractions in comparable concentrations. The corresponding fatty acids upon saponification or transmethylation of these fractions were the usual saturated acids in the less polar fraction, saturated and, additionally, unknown more polar acids in the middle fraction in a molar ratio of *ca* 1:1, and exclusively polar acids in the polar fraction. The saturated acids were 12:0, 14:0, 16:0, and 18:0 acids as identified by GC. The polar acids were β-hydroxy 12:0, 14:0, and 16:0 acids as revealed by MS and ^{1}H NMR. This means that in the three ester fractions the two hydroxyl groups of violaxanthin were either both esterified with the usual acids, or one hydroxyl with a usual acid and one hydroxyl with a β-hydroxy acid, or both hydroxyls with β-hydroxy acids, respectively. The β-hydroxy group itself was not esterified as was shown by acetylation. The minor xanthophylls of *Viola tricolor*, lutein, lutein epoxide, and neoxanthin, apparently showed corresponding ester patterns.

This is to our knowledge the first report of xanthophyll β-hydroxy acid esters from flower petals.

EXPERIMENTAL

Flower petals or lipid globules [6] from *Viola tricolor* L. subsp. *maxima* were extracted with acetone. Violaxanthin esters were separated by Si gel TLC using petrol–Et_2O–Me_2CO (10:2:1), free fatty acids with $CHCl_3$–EtOAc–HCO_2H (40:6:3), and fatty acid methyl esters with petrol–Et_2O (2:1). Saponification was performed using ethanolic KOH. Acetylation was done in pyridine using Ac_2O. Fatty acid methyl esters were prepared by transmethylation using NaOMe or by methylation using CH_2N_2. Methyl esters were separated by GC (column, 2.4 m × 3.2 mm, packed with 10% EGSSX, isothermal at 210°, carrier gas N_2 at 30 ml/min, FID).

EIMS (probe) 70 eV, *m/z* (rel. int.): 103, formed by α-cleavage to the hydroxy group (100), 74, formed by the McLafferty rearrangement triggered by the ester carbonyl group [7] (34). CIMS (NH_3, probe) 200 eV, *m/z* (rel. int.): 304 $[M + NH_4]^+$ from C_{16} methyl ester (7), 287 $[M + H]^+$ from C_{16} (2), 276 $[M + NH_4]^+$ from C_{14} (100), 269 $[M + H - H_2O]^+$ from C_{16} (4), 259 $[M + H]^+$ from C_{14} (40), 248 $[M + NH_4]^+$ from C_{12} (46), 241 $[M + H - H_2O]^+$ from C_{14} (66), 231 $[M + H]^+$ from C_{12} (19), 213 $[M + H - H_2O]^+$ from C_{12} (29), 209 $[M + H - H_2O - MeOH]^+$ from C_{14} (8), 181 $[M + H - H_2O - MeOH]^+$ from C_{12} (5).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.008 (1 H, *br s*, H_β), 3.716 (3 H, s, COOMe), 2.823 (1 H, *d*, J = 4 Hz, OH), 2.514 and 2.410 (AB-part of ABX-spectrum, 1 H each, CH_2 (α), J_{AB} = 16.2 Hz, J_{AX} = 3 Hz, J_{BX} = 9 Hz (X = H_β)), chemical shift and large value of J_{AB} is indicative for the α-position to the carbonyl), *ca* 1.53 (1 H), ca 1.43 (2 H) and *ca* 1.258 (*ca* 17 H, CH_2–CH_2), 0.880 (3 H, tr, $J \approx 6.8$ Hz, Me). Upon addition of D_2O to the solution the OH-signal disappeared and the multiplet signal of H_β became slightly sharper.

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A TRIGLUCOSIDE OF HECOGENIN FROM FRUITS OF *AGAVE CANTALA*

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Key Word Index—*Agave cantala*; Agavaceae; saponin; hecogenin triglucoside.

Abstract—The fruits of *Agave cantala* contain a steroidal saponin which is a glycoside of hecogenin with three molecules of glucose. Its structure has been established as α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 3)hecogenin.

A review of the literature showed that while leaves of different *Agave* species contain a number of steroidal saponins [1] no work has been reported on the fruits of these plants.

The blackish fruits of *Agave cantala* were therefore collected from Srinagar–Garhwal, and dried and powdered. The defatted powder was extracted with ethanol which was concentrated to give a cream-coloured syrupy mass, which was purified in the usual manner [2]. The light cream-coloured powder obtained on TLC showed the presence of one major component, which was finally purified by column chromatography.

The purified substance (**1**) had mp 240–243°, $[\alpha]_D$ +95.5° (*c*, 1% MeOH), and its IR spectra showed bands at 860, 900, 920 and 980 cm^{-1} indicating that ring F is closed and there is no sugar attached to C-26. On acid hydrolysis (2 N H_2SO_4) it yielded a genin (44.8%), identified as hecogenin by mmp and co-chromatography with an authentic sample. The hydrolysate after neutralization was deionized with ion-exchange resins [Amberlite IRA 400 and IR 120 (H^+)]. The solution thus obtained was concentrated to a syrupy mass which by PC, GLC (silyl derivative) [3] and HPLC showed only the presence of glucose. The molar ratio of aglycone:glucose was 1:3 based on genin weight.

In order to determine the sequence and mode of linkage of sugars **1** was completely methylated and this was confirmed by IR spectra. The permethylated saponin was subjected to methanolysis and the resulting methyl glycosides of the methylated sugars thus obtained were identified by GLC as 2,3,4,6-tetra-*O*-methyl-α-D-glucose (1 mol), and 2,3,6-tri-*O*-methyl-α-D-glucose (2 mol). On enzymic hydrolysis with β-glucosidase, no sugar was liberated indicating that the glucose molecules are α-linked. On treatment with $NaIO_4$ no free sugar was liberated, suggesting that all three glucose molecules are linked in such a manner that they are all oxidized by periodate. The molecular rotation calculations of the saponins based on Klyne's rule [4] also support the α-linkage of the sugars. The observed $(M)_D$ value, +874.78, is in reasonable agreement with the calculated $(M)_D$ value, +882.38.

Thus on the basis of above results **1** is identified as α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 3)hecogenin.

EXPERIMENTAL

All specific rotations are equilibrium values. Whatman filter paper No. 1 was used for PC: for sugars the solvents were *n*-BuOH–pyridine–H_2O (6:4:3) or EtOAc–pyridine–H_2O (22:10:1) and sprayed with *p*-anisidine HCl or alkaline $AgNO_3$. TLC of **1** was carried out on Si gel in *n*-BuOH–HOAc–H_2O (4:1:5) or $CHCl_3$–MeOH–H_2O (13:7:2). The sprays were 5% H_2SO_4 or cinnamaldehyde reagent. GC was carried out using 8% OS-138 on Chromosorb-W (NAW) column with N_2 and FID. HPLC was carried out using column—μ Bondapak/carbodydrate, solvent system acetonitrile–H_2O (4:1).

Isolation and purification of **1**. Dried and powdered fruits of *Agave cantala* collected from plants growing in Srinagar–Garhwal were defatted (petrol 40–60°), followed by