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A Convenient Method for Conversion of Flavonols into Anthocyanins

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Abstract: *Reduction of rutin by zinc amalgam in 3% absolute methanolic hydrochloric acid provides pure 3-O-β-D-(6"-O-α-L-(6"'-deoxy-mannosyl)) glucopyranosyl-cyanidin in good yield.*

The reductive synthesis[1] of highly hydroxylated and/or methoxylated flavylium cations, using the related flavonol molecules as precursors, has been accomplished either by reductive acetylation[2] or by reducing agents like LiAlH$_4$ and NaBH$_4$[3].

The use of zinc amalgam is here described as an alternative to the above mentioned methods. It presents a great stability in different solvents, such as methyl alcohol or water, and in a wide range of temperatures; moreover, it is easier to employ than sodium amalgam. Also, its redox potential is higher than that of aluminium amalgam[4]. The use of zinc amalgam requires a short reaction time and, since it is not soluble in the solvents used throughout the synthesis, it can be removed by a simple filtration, allowing thus a convenient procedure for the purification of the anthocyanin obtained.

In the present work, zinc amalgam was prepared as described in ref.5. Rutin (**1**), a flavonol glycoside, was reduced in 3% absolute methanolic hydrochloric acid to give the anthocyanin 3-O-β-D-(6"-O-α-L-(6"'-deoxy-mannosyl)) glucopyranosyl-cyanidin (**2**) (a natural anthocyanin, antirrhinin, first extracted from *Antirrhinum majus*[6]), with a final yield of 60% after purification (Scheme 1).
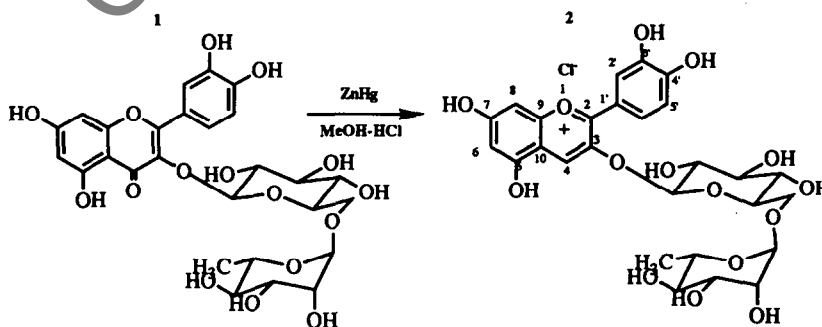
Scheme 1

- The reaction,which takes about 10 minutes until completion, was monitored by UV-visible absorption spectrophotometry by means of a Hewlett-Packard diode-array spectrophotometer.

We verified that zinc amalgam promotes the reduction of the flavylium cation if the time of contact with the reducing agent is too long. Nevertheless, the original anthocyanin can be almost quantitatively recovered by complementary stirring in air after removal of the zinc amalgam.

The anthocyanin obtained through the above procedure was first purified on Amberlite CG-50 (100-200 mesh) to separate different salts and then crystallized in a mixture of 1% absolute methanolic hydrochloric acid and diethyl ether to afford the pure pigment. The purity of **2** was checked by HPLC. The HPLC equipment consisted of a Spectra-Physics intelligent pump equipped with a Rheodyne 7125 injection valve (100 μl loop). A Lichrospher RP-18 column was used with an eluent system constituted of 5% solution A (HCO_2H, CH_3CN, H_2O (5:47.5:47.5)) in solution B (HCO_2H, H_2O (5:95)) and the effluent was monitored at 280 nm and 510 nm by means of a diode-array Hewlett-Packard detector.

^{1}H NMR and 2D-COSY (400MHz, $(CD_3)_2SO,CF_3CO_2D$) analysis confirm the purity and structure of the anthocyanin[7]. The FAB-MS of this pigment gave its molecular ion at 595.1 m/z [M^+], in good agreement with the mass calculated for $C_{27}H_{31}O_{15}$ (595.5).

The electronic absorption properties of **2** presented in Table 1 are in accordance with the data commonly reported for this kind of pigments[9]. The $\varepsilon_{(max)}$ value for the visible absorption band is low when compared with the most common anthocyanins, but is in accordance with the values determined in our laboratory for other 3-disaccharides[10].

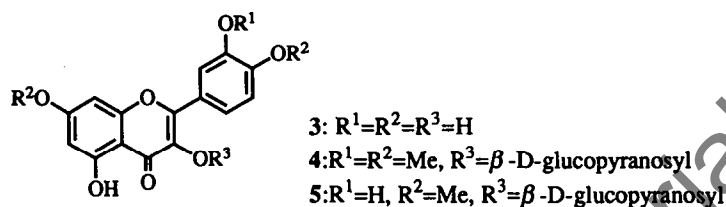**Table 1.** UV-visible characteristics of the flavylium cation form of 2. Solvent: H_2O/HCl; pH=0.9; T=25 °C.

$\lambda_{max,vis}$ /nm	$\lambda_{max,UV}$ /nm	$A_{440\ nm}/A_{max,vis}$	$\lambda_{max,vis}$ /nm with Al^{3+} §
510 (7000)	280 (6500)	0.44	555

§ pH=3.15. Values in parentheses are molar absorption coefficients in $mol^{-1}\ dm^3\ cm^{-1}$.

It is well known[11] that anthocyanins undergo hydration and deprotonation reactions in mildly aqueous solution, producing respectively the hemiacetal and quinonoidal base forms. Kinetic and thermodynamic measurements, performed on the UV-visible apparatus according to a procedure described elsewhere[10], yielded characteristic values for the constants of the hydration and deprotonation reactions ($pK_h=2.93\pm0.02$ and $pK_a=3.58\pm0.03$)[12] .

This catechol-type anthocyanin is a good support for complexation[13] with small highly charged metallic ions such as Al^{3+} (Table 1), Mg^{2+} or Fe $^{3+}$. This complementary effect of molecular copigmentation[14] partially prevents the nucleophilic attack of water to positions 2 or 4 of anthocyanins with the consequent formation of undesirable colourless forms (hemiacetal and chalcone).

Other synthetic (5-hydroxy-3',4',7-tri-O-methyl-3-O-β-D-glucopyranosyl-flavonol (**4**) and 3',5-dihydroxy-,4',7-di-O-methyl-3-O-β-D-glucopyranosyl-flavonol (**5**)) or natural flavonols (quercetin (**3**)) (Scheme 2) have already proved to be good substrates for reductive conversion, by zinc amalgam, into flavylium cations[15] signalling thus a wider utilization of this practical and simple procedure as a method of choice to convert an extensive range of flavonols into the corresponding anthocyanins. The corresponding anthocyanins were obtained in pure state respectively with an yield of 55% (**4**), 52% (**5**) and 36% for quercetin (**3**).

3: $R^1=R^2=R^3=H$
4: $R^1=R^2=Me$, $R^3=\beta$-D-glucopyranosyl
5: $R^1=H$, $R^2=Me$, $R^3=\beta$-D-glucopyranosyl

Scheme 2

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7. ^{1}H NMR (400MHz, DMSO-d_6 -CF$_3$CO$_2$D / *: assigned by ^{1}H-^{1}H COSY) δ ppm (*J* in Hz) (aglycone) 8.80 (1H, s, H4), 8.20 (1H, dd, *J* = 9; 2.1, H6'), 7.03 (1H, d, *J* = 9.1, H5'), 8.00 (1H, d, *J* = 2.0, H2'), 6.90 (1H, d, *J* = 2.0, H8), 6.78 (1H, d, *J* = 2.0, H6); (glucosyl) 5.26 (1H, d,*J* = 8, H1), 3.53 (1H, t, *J* = 7.5, H2*), H3 not assigned, 3.48 (1H, m, H4*), 3.17 (1H, m, H5*), 3.42 (1H, dd, *J* =

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_10.6; 7.5, H6a*), 3.91 (1H, dd, J = 10.6; 7.5, H6b*), (rhamnosyl) 4.52 (1H, d, J = 3.1, H1*), 3.61 (1H, m, H2*), H3, H4 not assigned (complex signals), 3.40 (1H, m, H5*), 1.15 (3H, m, methyl*). Protons 6 and 8 were attributed according to ref. 8.

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