

SIMPLE SYNTHETIC PROCESSES FOR CHRYSIN, NORWOGONIN AND THEIR DERIVATIVES

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ABSTRACT

A simple and convenient synthesis of chrysin and Nor wogonin is reported.

KEYWORDS: Chrysin, Nor wogonin, 5,7-Dihydroxy flavone, 5,7,8-trihydroxy flavone, 5-hydroxy-7-O-benzyl flavone, 5,8-dihydroxy-7-O-benzyl flavone, 5-hydroxy-7-O-benzyl-8-O-benzoyl flavone, nuclear oxidation

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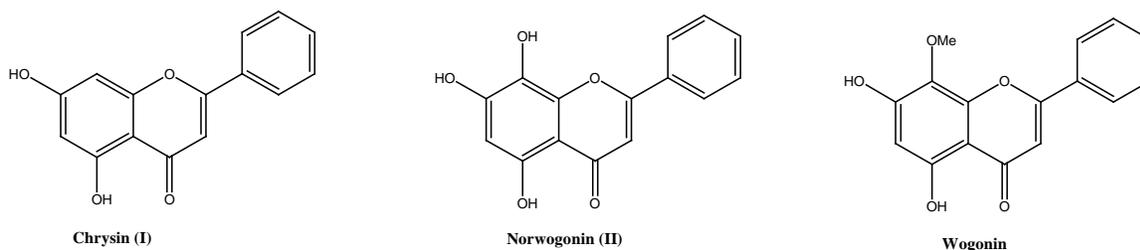
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INTRODUCTION

Among the various groups of secondary plant metabolites, polyhydroxy flavones have been reported to possess numerous pharmacological activities such as anti-cancer, anti tumor, anti-inflammatory, anti oxidant etc¹⁻⁵. Chrysin, Wogonin, Baicalein, Quercetin etc. are among the polyhydroxy flavones who also show inhibitory activities against iNOS cox-2 and PGE 2⁶. Wogonin and norwogonin are structurally related flavones and respectively contain OH and OMe groups at C-8 position. According to literature, Norwogonin is more effective than wogonin in inducing apoptosis in ML-60 cells. Chrysin also shows anti cancer activity in the same manner⁷. Synthesis of Wogonin is reported many where but that of Norwogonin is rare. In our ongoing synthetic studies on the flavonolignans⁸, we needed Chrysin, Norwogonin and their O-benzyl derivatives as some key intermediates. We report herein the synthesis of these intermediates and also their mutual transformations where ever possible for structural confirmation.



MATERIALS & METHODS

Melting points are uncorrected. ¹H NMR spectra were recorded on a Varian EM-300 (60 MHz) spectrometer in CCl₄/CDCl₃ solvent using TMS as internal standard. IR spectra were recorded in a Nicolet FT-IR spectrometer (Model Impact 400) using KBr pellets. GC/MS was carried out on a Fisons instruments TRIO-1000.

5, 7-Dihydroxyflavone (Chrysin) (I)

Phloracetophenone(5g)(oven dried at 120°C overnight), anhydrous K₂CO₃(freshly ignited and cooled in a desiccator)(50g), dry acetone(200mL) and benzoyl chloride(12mL) were stirred vigorously under reflux with the aid of a mechanical stirrer. The red colour of the solution faded as the reaction proceeded and finally became colourless. After 20hr, acetone was evaporated completely and H₂O(400mL) was added to it. A yellowish solid precipitated which was filtered and washed with H₂O(10mL). After drying at 60°C under vacuum for 15hr, the solid was recrystallised from EtOH to give yellowish crystal (I) melting at 275 – 276 °C (lit¹⁰ mp 275 – 276 °C). Yield 3g

EIMS (m/z, %): 255(M⁺ +1, 22.9), 254 (M⁺, 100.0), 226 (17.6), 152 (23.4), 124 (23.8), 113 (14.6), 105 (9.7), 102 (7.8), 77 (14.4).

5, 7-Diacetoxyflavone (Ia)

The above solid(0.2g) was dissolved in freshly distilled acetic anhydride(2mL) and was heated on a water bath with 2 drops of pyridine for 3 hr. Crushed ice was added and the diluted with water and then left for 1 hr. The precipitated solid was filtered, washed with little water and dried at 60°C under vacuum for 15hr to give 0.2g of the crude solid which was recrystallised twice in EtOH to give the diacetate as colourless plates melting at 191 -192 °C (lit¹⁵ mp 192 -193°C).

7-Benzyloxy, 5-hydroxyflavone (III):

Chrysin (I, 2.3 g, 0.0090 mol), benzyl chloride (1.26 g, 0.0099 mol), anhydrous NaHCO₃ (3.8 g, 0.0452 mol) were refluxed in 50 mL dry acetonitrile for 20 h. The reaction mixture was filtered with hot acetone washings and the filtrate was evaporated to dryness at 50 °C under reduced pressure to give a brownish viscous mass (2.86 g, 92 %) which solidified later melting at 177 °C (lit.¹⁶ m.p. 177-178 °C) and was characterized to be 7-O-benzyl chrysin (II). It gave a positive FeCl₃ test.

IR (KBr, ν_{\max} cm^{-1}): 1058, 1595, 1645, 3450 cm^{-1} .

^1H NMR (CDCl_3 , δ): 5.2 (s, 2H, $-\text{CH}_2\text{-Ph}$), 6.6 (s, 1H, H-6), 6.7 (s, 1H, H-8), 6.8 (s, 1H, H-3), 7.5 (m, 8H, H-3', H-4', H-5' and $-\text{CH}_2\text{-Ph}$), 8.0 (m, 2H, H-2' and H-6'), 12.5 (s, 1H, $-\text{OH}$).

EIMS (m/z, %): 344 (M^+ , 11.0), 209 (11.4), 119 (14.6), 105 (15.4), 91 (35.2), 84 (10.6), 77 (15.3), 65 (4.3), 40 (100), 49 (11.7).

8-Benzoyloxy, 7-benzyloxy, 5-hydroxyflavone (IVa)

In a 250mL RB flask, 7-O-Benzyl chrysin (II)(1.2g) was dissolved in 20 mL pyridine and KOH solution(2g in 30 mL H_2O) was added to it. Pyridine (20mL) was added further to clear the turbidity obtained and $\text{K}_2\text{S}_2\text{O}_8$ solution(1.8g in 60mL H_2O) was added drop wise from a dropping funnel during 2 hr while stirring. During the addition 10mL pyridine was added each time turbidity appeared(total pyridine added= 100mL) and the stirring was continued for 24 hr at RT. Reaction mixture was kept over ice bath and made acidic to pH 4 with slow addition of conc.HCl(105 mL Conc. HCl consumed) with stirring during which unreacted II was precipitated. It was filtered (0.35g) and the mother liquor was extracted with diethyl ether (3 x 50mL) to remove additional unreacted II from the solution. The aqueous portion was again made acidic to pH 2 by 20mL conc.HCl and then solid Na_2SO_3 (5g) was added to it. It was heated under stirring over a preheated water bath for 30 min during which yellowish solid precipitated. It was cooled to RT and filtered. The crude solid was recrystallised with EtOH to furnish yellowish needles (IVa) melting at 245°C. It was +ve to DNP test. Yield 0.15g

IR (KBr, ν_{\max} cm^{-1}): 1060, 1599, 1660, 1705, 3460.

^1H NMR (DMSO-d_6 , δ): 5.3 (s, 2H, $-\text{CH}_2\text{-Ph}$), 6.8 (s, 1H, H-6), 7.6 (bs, 14H, H-3, H-3', H-4', H-5', $-\text{OCO-Ph}$ and $-\text{CH}_2\text{-Ph}$), 8.2 (m, 2H, H-2', H-6'), 12.4 (s, 1H, $-\text{OH}$).

EIMS (m/z, %): 464 (M^+ , 16.2), 374 (23.7), 373 (82.9), 272 (4.2), 129 (10.4), 105, (100.0), 91 (64.6).

7-Benzyloxy, 5,8-dihydroxyflavone (IV)

The greenish yellow solid (IVa)(0.1g) was dissolved in 10% aq/alcoholic NaOH solution(15mL)(2.5g of NaOH dissolved in 5 mL H_2O and to that 20mL EtOH was added) forming a reddish brown solution. It was refluxed on water bath for 30 min, after which crushed ice was added and acidified with 10% HCl. The solution turned yellow and was extracted with EtOAc which upon evaporation afforded 0.06g of crude solid which was recrystallised from EtOAc/Hexanes to give yellow solid (IV) melting at 185°C (lit ¹² mp 184-185°C). It was +ve to FeCl_3 test(intense greenish blue colour) having 0.2 R_f value and also negative to DNP test, where as starting (IVa) gave faint blue colouration to FeCl_3 test and $R_f = 0.6$.

IR (KBr, ν_{\max} cm^{-1}): 1058, 1600, 1657, 3465.

^1H NMR (CDCl_3 , δ): 5.3 (s, 2H, $-\text{CH}_2\text{-Ph}$), 6.7 (s, 1H, H-6), 7.32 (s, 1H, H-3), 7.5 (s, 5H, $\text{CH}_2\text{-Ph}$), 7.6 (m, 3H, H-3', H-4', H-5'), 8.0 (m, 2H, H-2', H-6').

EIMS (m/z, %): 360 (M^+ , 4.2), 332 (4.2), 269 (6.8), 258 (8.6), 241 (4.3), 167(7.1), 139(7.9), 129 (9.9), 105 (64.3), 91 (55.4).

5, 7, 8-trihydroxyflavone (Nor-Wogonin) (II)

The yellow solid (IV) (0.2g) was taken in glacial acetic acid (10mL) and Conc. HCl (2mL) and the mixture was heated in a boiling water bath for 2 hr. The acetic acid and benzyl chloride were distilled out under vacuum and crushed ice was added to the residue. The bright yellow solid was filtered, washed with water which was recrystallised with EtOH to give golden yellow coloured crystal(II) melting at 258 °C (lit ¹⁴ mp 258-260°C). It was +ve to FeCl_3 test(greenish colouration). Yield 0.13g

IR(KBr, ν_{\max} cm^{-1}): 1058, 1562, 1670, 3460.

^1H NMR (CDCl_3 , δ): 6.75 (s, 1H, H-6), 7.4 (s, 1H, H-3), 7.7 (m, 3H, H-3', H-4', H-5'), 8.1 (m, 2H, H-2', H-6').

EIMS (m/z, %): 270 (M^+ , 24.8), 241 (18.5), 167 (16.8), 139 (8.6), 129 (10.5), 107(17.6), 105(57.7), 102 (9.8).

Acetylation of (II)

Norwogonin (**II**) prepared above (0.1g) was heated with acetic anhydride(10 mL) and a drop of pyridine in a water bath for 3 hr .Crushed ice was added and the solid was filtered, washed with water and dried at 60 °C under vacuum which was recrystallised with EtOAc to give colourless crystals (**IIa**) melting at 225 °C (lit ¹⁴ mp 225-226 °C).Yield 0.1g

Deacetylation of (IIa)

Norwogonin triacetate (**IIa**) (0.1g) was heated with EtOH(10mL) and Conc.HCl (10mL) on a boiling water bath for 3 hr.The solvent was recovered under reduced pressure and the crude product was recrystallised with EtOH to give golden yellow crystals melting at 259 °C.It matches in all respect with the Norwogonin made earlier.

Benzylation of (II)

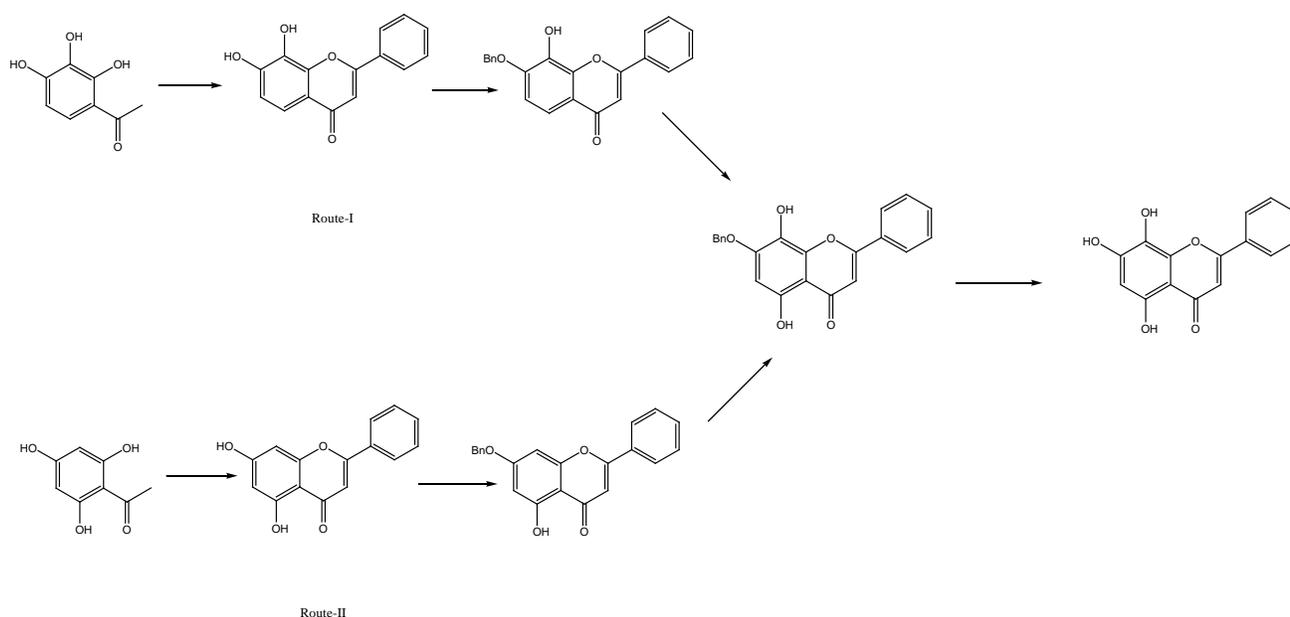
A mixture of Norwogonin(**II**) (3g), acetone (270 mL), EtOH (10 mL), Benzyl chloride(1.26mL, 1 mol), NaHCO₃ (9g) and NaI (1.5g) was refluxed for 30 hr.After filtration and washing of residue with acetone, the solvent was recovered yielding a light yellow solid which was recrystallised from EtOAc/hexanes to furnish yellow needles having mp 183 °C which matched with the authentic sample.

RESULTS AND DISCUSSIONS

The main target of this study is the preparation of Norwogonin. It can be synthesized either by

- Reaction of benzoic anhydride with 2, 3, 4, 6-tetrahydroxy acetophenone or
- Preparing dihydroxy flavone(either 7,8-dihydroxy or 5,7-dihydroxy) and subjecting them to persulfate oxidation.

There is a possibility of ring isomeric change during flavone synthesis if we opt for plan A. For example, when anisic anhydride and sodium anisate were condensed with 2, 4-dihydroxy-3, 6-dimethoxy acetophenone, the reaction was accompanied by the ring isomeric change and 5, 7-dihydroxy-6, 4'-dimethoxy flavone was obtained ⁹.Plan B is outlined in the scheme- I. Out of the two routes, the route-I was not interesting as the nuclear oxidation step gave negligible percentage of product .Hence we started exploring the route-II.

**Scheme-I**

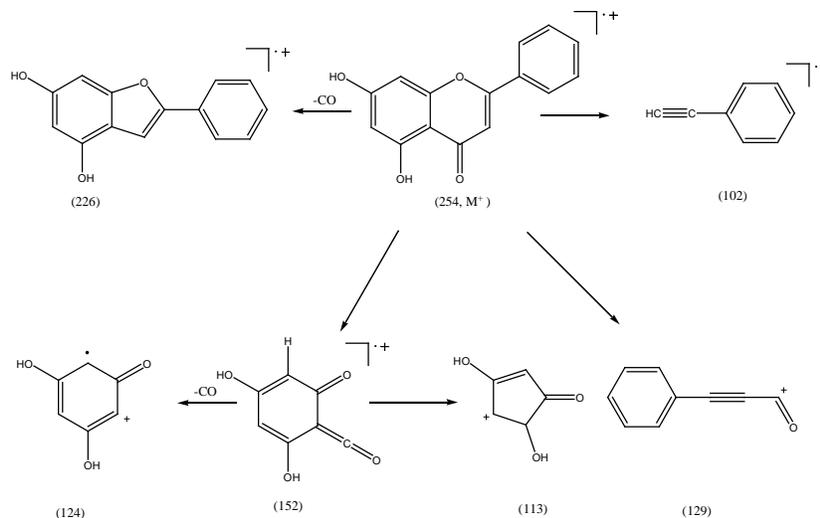
We encountered a serious problem during nuclear oxidation step here also. The beauty of flavonoid chemistry is the reactivity of various hydroxyl groups using which one can alkylate and dealkylate at various hydroxyl groups selectively. Since 7-OH group in 5, 7, 8-trihydroxy flavone is more reactive for alkylation as compared to OH group in other position, it can initially be protected by a suitable group like benzyl, thus leaving the hydroxyl group in 5 and 8 position free. Based on the considerations that between these two OH groups, the OH group in 5 positions will be in chelation with the flavone carbonyl group and hence the 8-OH group would undergo alkylation easily, the present synthesis of nor wogonin using benzyl group for the protection of the 7-OH group was undertaken.

Synthesis of Chrysin (I)

Chrysin (5, 7-Dihydroxyflavone) was synthesized by a modification of the method proposed by Rama Rao *et al*¹⁰. The previous method was a two step reaction starting from phloracetophenone in which the first step yielding a mixture of the diketone and the flavone which was then subjected to reflux in the presence of aqueous K_2CO_3 for effecting the complete conversion to flavone. In the modified procedure used here, phloracetophenone was vigorously stirred with excess benzoyl chloride in the presence of anhydrous K_2CO_3 and dry acetone with the aid of a mechanical stirrer for 20 hr. After filtering the reaction mass, the acetone was concentrated and the crude solid was crystallized from ethanol to give a yellowish solid (I) matching with the m.p. of Chrysin *ie.* 275 – 276 °C.

The EI mass fragmentation of (I) exhibited the expected peaks apart from 255 ($M^+ + 1$), 105 ($Ph-C=O^+$) and 77 ($C_6H_5^+$) as shown below.

The EI mass fragmentation of (I)



(I) was further characterized by acetylation with Ac_2O /pyr to get the diacetate. m.p. 191 – 192 °C.

Monobenylation of chrysin

When Chrysin was subjected to benzylation using benzyl chloride in K_2CO_3 and acetone, a mixture of mono and bis benzyl Chrysin was obtained. We developed successfully a method to get exclusively mono benzylated product (II) with 95 % yield although we were able to debenzylate selectively to get the mono product (III) from the bisbenzyl chrysin obtained earlier¹¹.

Persulfate oxidation of (III)

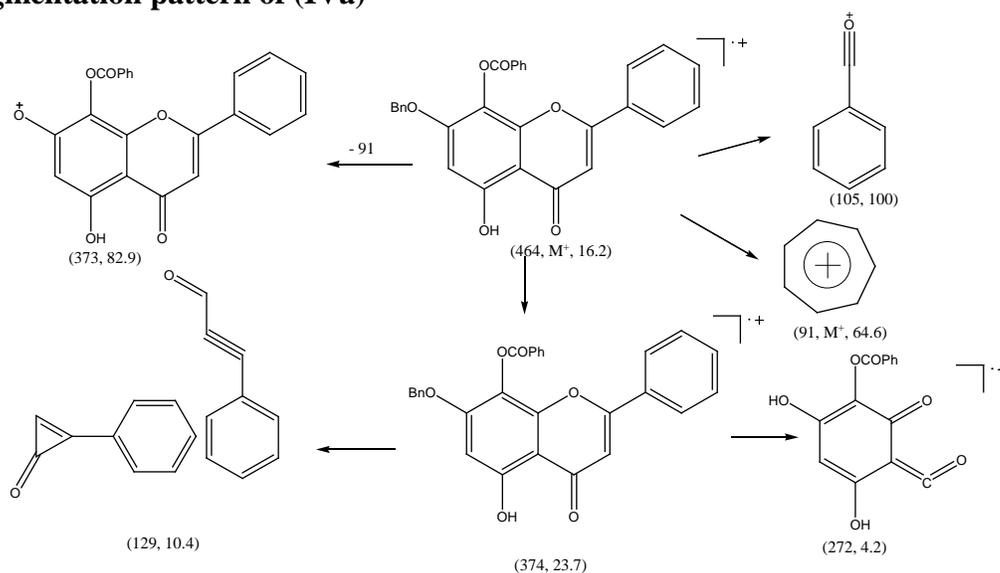
The nuclear oxidation of (III) was carried out with potassium persulfate – a standard procedure in the flavonoid field. It is generally carried out by slow addition of persulfate solution to a stirred solution of the compound in 10 % aqueous alkali with or without pyridine. Selective removal of unchanged starting material at congo red pH followed by hydrolysis with conc. HCl in the presence of sodium sulfite leads to the desired product. This procedure was applied to Chrysin (I), but negligible % of the desired product was obtained and most of the unreacted starting materials were isolated back. Hence this was attempted with 7-O-benzylated Chrysin (III).

Different conditions were tried for the optimization of the above procedure to improve the yield. As (III) was practically insoluble in aqueous alkali, pyridine was employed to make it soluble and was found that for 1g of starting flavone, 100 mL of pyridine was essential to give a better yield. Lowering the amount of pyridine resulted in recovering most of the starting flavone during congo red acidification. In one experiment instead of adding persulfate solution to flavone in alkali, both the alkali and persulfate solution were added simultaneously to (III) dissolved in pyridine. Although the same product was obtained with a very low yield, most of the starting material was recovered during acidification of the reaction mixture to pH 4.

Following the optimized procedure, the product obtained as an yellowish solid (IVa). It had a lower R_f value (0.4) than 7-O-benzyl chrysin(III) (0.7) in benzene solvent system and it gave a faint colour to $FeCl_3$. It melted at 245 °C, whereas the lit¹² m.p. of the desired flavone (IV) is 185 °C. Its IR spectrum showed a peak at 1705 Cm^{-1} attributable for a carbonyl group. In its $^1\text{H NMR}$ spectrum, the two proton singlet due to benzylic protons appeared at 5.3 δ . The aromatic proton, H-6 was observed as one proton singlet at 6.8 δ and H-2' & H-6' were observed as two proton multiplet at 8.2 δ . However, the multiplet at 7.6 δ integrated for fourteen hydrogens instead of the expected nine hydrogens. One hump at 12.4 δ was assigned to the hydroxyl proton.

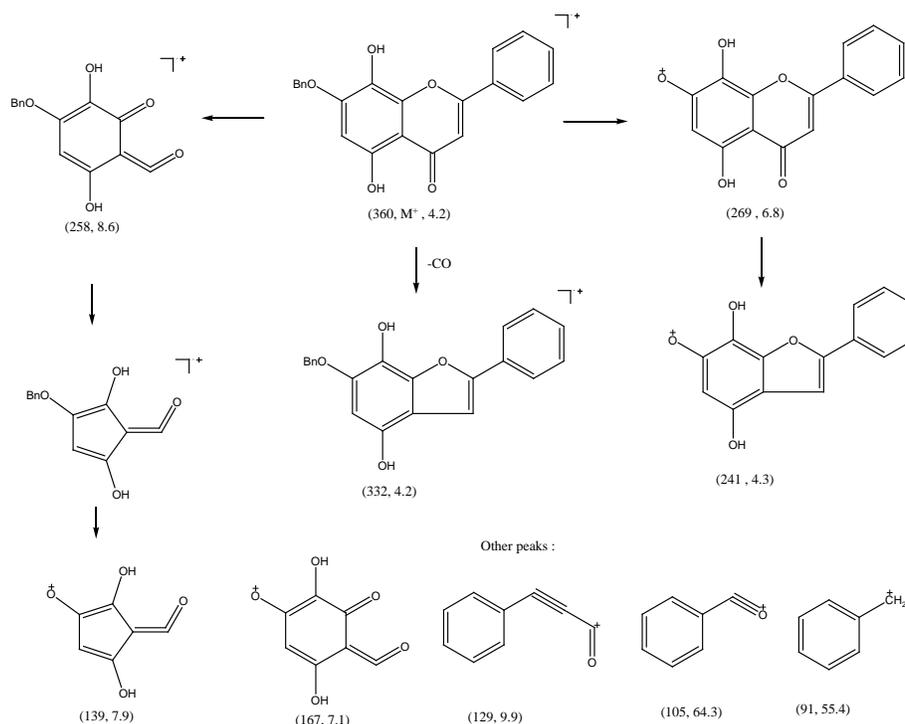
EI mass spectrum of the above revealed that the molecular weight to be 464. From this and the analysis of the fragment ions, the constitution of the product was established to be (IVa). This is a very unusual behaviour in persulfate oxidation of flavones.

EI mass fragmentation pattern of (IVa)



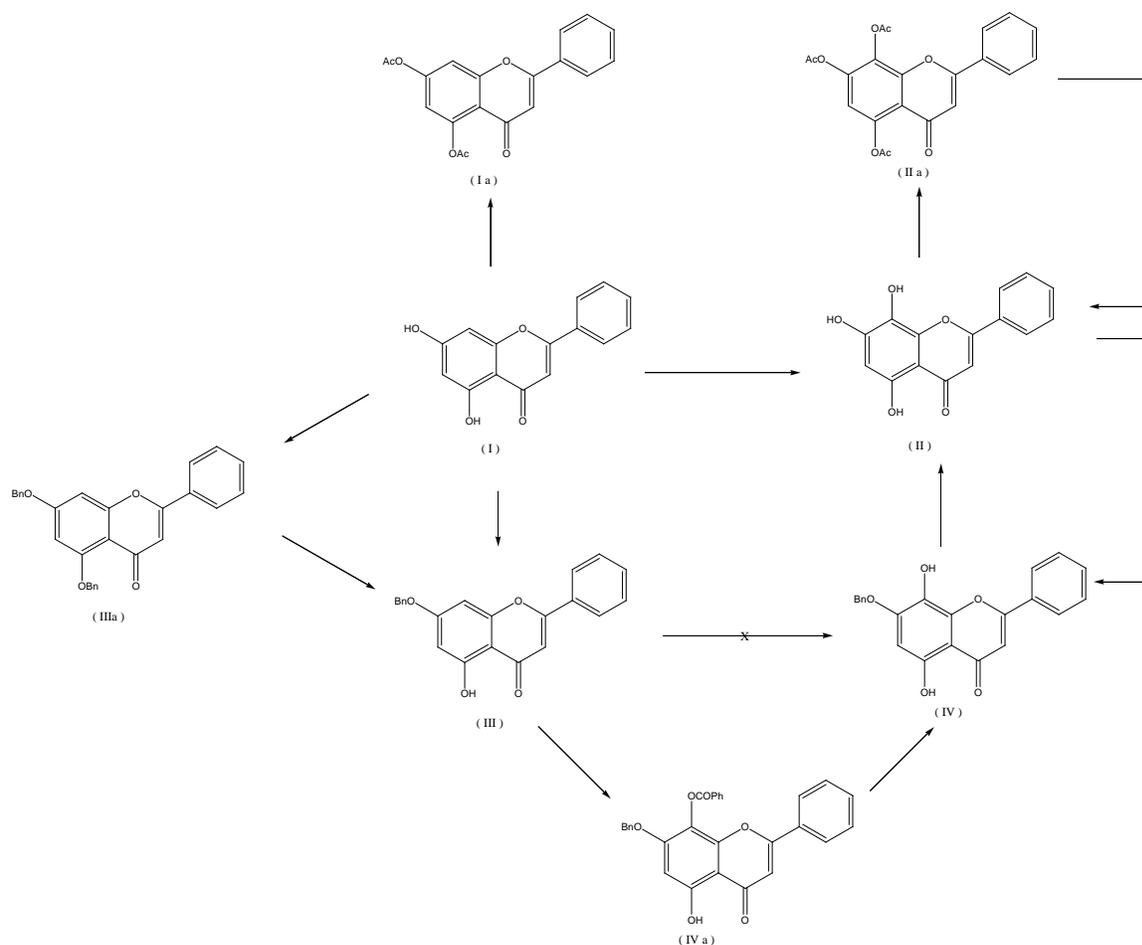
Alkali hydrolysis of (IVa)

In order to remove the unwanted benzoyl group, (IVa) was refluxed with 10 % aqueous/alcoholic NaOH solution and the product after recrystallisation from EtOH afforded yellowish solid (IV) melting at 185 °C which matched with the m.p. of desired flavone¹². Unlike (IVa), it gave intense greenish blue in FeCl₃ test. Its IR spectrum lacked the carbonyl peak at 1705 cm⁻¹. Apart from other peaks, the aromatic region integrated for twelve protons indicating the absence of the benzoyl group. Finally the product was confirmed from the EI mass spectrum and its fragmentation pattern is as shown below.

EI mass fragmentation pattern of (IV)

Earlier the mp of this was reported to be 220°C by Rao *et al*¹³ which was revised by Bhardwaj *et al*¹² to be 185°C. The mp 220°C could be attributable for a product containing a mixture of (IVa) and (IV).

Finally, 7-Obenzyl norwogonin (IV) was debenzylated to get Norwogonin (II) in 80 % yield. Its m.p. (258 °C) matched with that reported in literature¹⁴. In order to confirm the structure further, mutual transformations were also carried out. The prepared Norwogonin (II) was acetylated to get the corresponding triacetate (V) and the triacetate (V) was also deacetylated to get back Norwogonin (II) which was again benzylated to get 7-Obenzyl norwogonin (IV). All the products matched in all respect with the data of those products prepared earlier. The summary of the work is outlined in scheme-II.



CONCLUSION

We have developed a very simple and convenient process for the synthesis of chrysin and Nor wogonin which will be very useful in flavonoid chemistry. Besides this we have also reported the simple synthetic procedures for their acetyl and benzyl derivatives.

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