SYNTHESIS AND EVALUATION OF RESVERATROL FOR ANTICANCER ACTIVITY

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ABSTRACT

Resveratrol was synthesized using appropriate synthetic route. The structure of the compound were established by means of TLC, IR and ^{1}H-NMR and elemental analysis. Synthesized Resveratrol was evaluated for anticancer activity by using onion route tip method. Compound showed significant activity as comparing with that of standard, cytotoxic agent.

1.INTRODUCTION

5-alkyl Resorcinol's are naturally occurring compounds, present in many plants. Their 5-alkenyl derivatives, primarily those with stilbene structure, showing interesting antileukemic activity. Resveratrol is also one of them. Several attempts have been made to synthesize di and trihydroxy stilbenes (Alonso, 1997;Jeandet,1996). In 1997 Alonso synthesized Resveratrol using 3, 5 dimethoxy benzyl alcohol as the starting material. Synthesis of Resveratrol was also taken up so as to standardize the scheme for its chemical synthesis. The percentage of this trihydroxy stilbene, in red wine is from 2.861 to less than 0.001 Mmol/L and in white wine from o.438 to less than 0.001 Mmol/L. Since its quantity in natural sources is very low, there is a need to improve the yield of Resveratrol and hence synthesis of Resveratrol is undertaken in our present study.

2.MATERIALS AND METHODS

The chemicals used were of AR grade. The progress of reaction and purity of products were analyzed by TLC. Melting points were taken in an open capillary tube and are uncorrected. IR spectra were obtained on Bruker FTIR. ^{1}H-NMR spectra were recorded on Bruker advance II 400 in DMSO *d6* by using TMS as an internal standard.

ANTICANCER ACTIVITY:

Anticancer activity was done by using Onion Root Tip Method (Hoda,1992). The concentrations of the drugs are made by suspending the drug in 0.5%v/v Tween 80 in distilled water. The different concentrations used are 25, 50, 100 and 200 µg/ml. The standard is also prepared in the same manner at the concentrations of 25, 50, 100 and 200 µg/ml. However , with the increase in the dose of the drugs, there is a corresponding decrease in the mitotic index values for all the above mentioned compounds.

Onions of good quality are rooted in water and the roots are treated with drugs for 24 h. The root tips around 2 to 3mm, are cut and fixed in acetic acid : alcohol (1:3). Root tip squash is made, observed under microscope and mitotic index is calculated (Aprem and Handique,1997).

Procedure

Fresh disease free onions of uniform size are taken, base stem portions are slightly scraped and roots are allowed to grow in bankers containing water. The onions are rooted for about 2-3 cm within five days. On the sixth day the rooted onions are treated with respective drugs for 24 h. On the day seven the roots are washed with water and six root tips are collected and fixed in acetic acid : alcohol (1:3) for half an hour. The roots tips are warmed for few seconds in a watch glass containing a mixture of acetocarmine stain with 1N HCl (9:1). The stained root tips are then squashed and observed under microscope (45 ×) and the number of dividing cells versus non-dividing cells are counted. Then mitotic index is calcualated using the formula, Mitotic Index = Number of dividing cells/ Number of non dividing cells.

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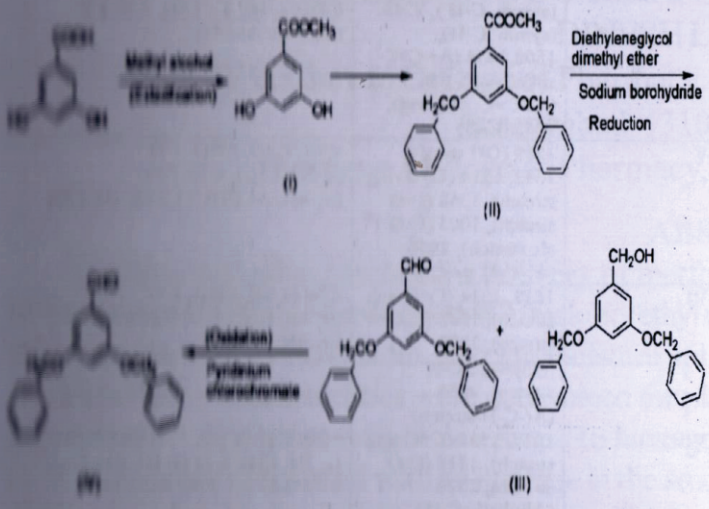
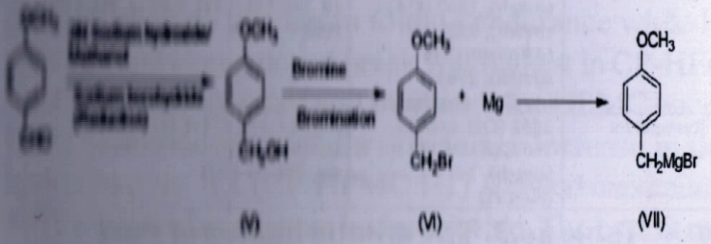
SCHEME:

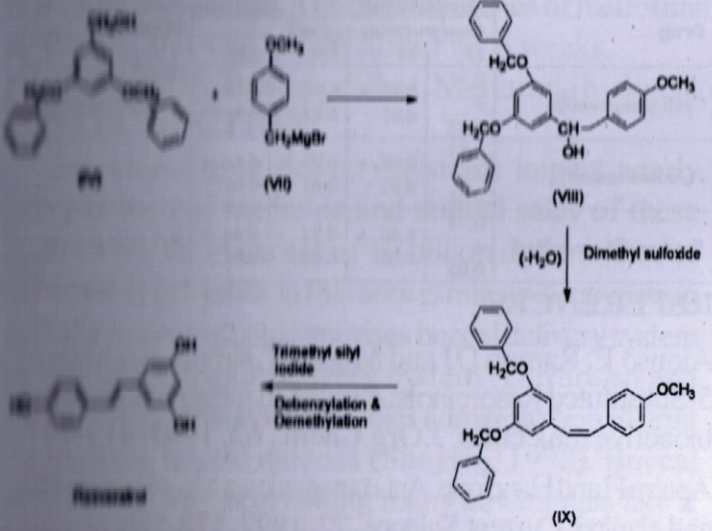
Step-I: Preparation of intermediate IV



Step-II: Preparation of methoxybenzyl magnesium bromide (VII)



Step-III: Preparation of Resveratrol



PROCEDURE

Preparation of intermediate III :

A mixture of 1-methyl 3,5 dimethoxy benzoate 20g, Diethylene glycol dimethylether 57.2 ml and Aluminium trichloride 8.04 g are taken in around bottom flask and 2.28 g of sodium borohydride is added slowly over a period of an half an hour and stirred at room temperature for two hours. Since this reaction is incomplete, heating is continued for another three hours at 50° C. Completion of this reaction is confirmed by TLC.

Preparation of intermediate IV:

Chromium (IV) oxide 20 g (0.2 moles) is rapidly added to 6M Hydrochloric acid 37 ml and stirred for five minutes. The homogeneous solution is cooled to 0°C and Pyridine 16 g (0.21 moles) is added dropwise over ten minutes. The reaction contents are recooled to 0°C, filtered through sintered glass funnel and dried for one hour in vaccum. Yellowish orage solid is obtained.

Preparation of (4-methoxyphenyl) methanol (intermediate V):

The solution of p-anisaldehyde 2.5 g in Methanol 200ml, is treated dropwise with the solution of sodium borohydride 3g, 2N sodium hydroxide 4ml and distilled water 35 ml, stirred at 0°C for one hour. The completion of the reaction is confirmed by TLC. The reaction mass is concentrated to decrease the volume of methanol to 50ml. The residue is treated with a solution of 5% sulfuric acid 20 ml (to make acidic to the blue litmus) and water 200ml. Extracted this reaction mass with chloroform. The Chloroform extrat is dried over anhydrous sodium sulphate and chloroform is removed under pressure.

Preparation of 1-(bromomethyl)-4-methoxybenzene (intermediate VI):

The solution of phosphorous tribromide 4.8 ml Tolune 10 mlis added dropwise over a period of 30 minutes to the solution of anisyl alcohol 18 g (130 Mmoles) in Tolune 100 ml. The mixture is treated with 3ml of pyridine, stirred for one hour. The completion of reaction is confermed by TLC. Poured this reaction mass in ice water 30ml, quenched, removed the organic layer, washed with 5% Hydrocloric acid 50 ml and dried over anhydrous sodium sulphate. The solvent is removed under pressure.

Preparation of (4-methoxy benzyl)magnesium bromide (intermediate VII):

A small volume of solution of methoxy benzyl bromide 3.16 in Tetrahydrofuran is added to a mixture of magnesium 430 mg (previously washed with petroleumether and then dried in oven for overnight at 103°C), Terahydrofuran 25 ml and one pellet of Iodine. The reaction is initiated with change in colour from brown to pink. After complete addition of bromide, the reaction mixture is stirred for one hour and methoxybenzyl magnesium bromide was obtained.

Preparation of Intermediate VIII:

A small volume of solution of methoxy benzyl bromide 3.16 g in Tetrahydrofuran is added to a mixture of magnesium 430 mg (previously washed with petroleumether and then dried in oven for overnight at 103°C), Tetrahydrofuran 25 ml and one pellet of iodine. The reaction is initiated with change in the colour from brown to pink. After the complete addition of bromide, the reaction mixture is stirred for one hour. Further, a solution of aldehyde (IV) 5 g in Terahyrofuran 10 ml is added dropwise over 30 minutes and stirred for one hour. The reaction is monitored by TLC. As the reaction mixture showed many impurities, the residue is chromatographed on silica gel with 50% benzene in hexane as solvent. Appropriate fractions are combined and evaporated and the brown coloured intermediate VIII is recovered.

Preparation of intermediate IX:

Dimethyl Sulfoxide 60 g is added to intermediate VIII 3 g and refluxed at 160-185°C for 18 h. The Completion of the reaction is confirmed by TLC. The reaction mixture is washed with water (4 X 200 ml), extracted with ethyl acetate. The ethyl acetate extract is dried over anhydrous Sodium sulphate and the solvent is removed under pressure. Brown paste of intermediate IX is recovered.

3.RESULTS AND DISCUSSION

An attempt is made to synthesize Resveratrol following the proposed protocol and it is possible to recover all the intermediate with Resveratrol which is synthesized by another route of synthesis. All the intermediates formed along with their % yields are given.

There is no siginificant difference in antimitotic activity amongst pure Resveratrol, synthesized Resveratrol and standard cytotoxic agent, cyclophosphamide, at a concentration of 50 µg/ml, 100 µg/ml, 200 µg/ml. However, with the increase in the dose of drugs, there is a corresponding decrease in the mitotic index values for all the above mentioned compounds.

Table no.1. Physicochemical Properties of the Intermediate compound:

Compound	Melting point (m.p.) (°C)	Yield (%)	Molecular formula
Intermediate IV	205°C	63.86	$C_{21}H_{18}O_3$
Intermediate V	223°C	85.68	$CH_3OC_6H_4CH_2OH$
Intermediate VI	240°C	80.00	C_8H_9OBr
Intermediate VIII	195°C	45.52	$C_{29}H_{27}O_4$
Intermediate IX	231	35.71	$C_{29}H_{26}O_3$

Table no. 2. Spectral Data of synthesized compound:

Intermediate	IR (KBr) v cm^{-1}	^{1}H NMR δ (ppm)
IV	1734 (C=O), 2926 (assym. C-H), 2745 (symm. C-H), 1508,1454 (Ar-C=C streaching), 738 (Ar-C=C, bending), 1053 (C-O)	5.20, 4.79 (s, 6H, 3 × CH_2), 6.21, 6.26 (s, 1H, 3 × CH), 7.19-7.27 (m, 10H, Ar-CH),
V	3389 (OH stret.), 1612, 1514 (C=C ring stretch), 1248 (C-O stretch), 1005 (C-O 1^0 alc stretch), 2956 (Ar-C-H stretch)	4.79 (s, 2H, CH_2), 3.73 (s, 3H, CH_3), 6.70-7.34 (m, 4H, Ar-CH), 11.0 (s, 1H, OH)
VI	1612, 1514 (C=C ring stretch), 1248 (C-O stretch), 2850 (Aliphatic C-H stretch), 2956 (Ar-C-H stretch)	4.59 (s, 2H, CH_2), 3.73 (s, 3H, CH_3), 6.60-7.14 (m, 4H, Ar-CH)
VIII	1618, 1575 (C=C ring stretch), 1218 (C-O stretch), 2876 (Aliphatic C-H stretch), 3014 (Ar-C-H stretch), 3374	5.20, 3.15 (s, 6H, 3 × CH_2), 4.89 (s, 1H, CH), 3.73 (s, 1H, CH_3), 6.72-7.34 (m, 17H, Ar-CH)
IX	1633, 1567 (C=C ring stretch), 1362 (C-O stretch), 2891 (Aliphatic C-H stretch), 2985 (Ar-C-H stretch)	5.23 (s, 4H, 2 × CH_2), 6.56 (d, 2H, CH=CH), 3.65 (s, 3H, CH_3), 6.82-7.32 (m, 17H, Ar-CH)
Resveratrol	3452 (OH stretch), 1653, (CH=CH stretch), 3017 (Ar-CH)	6.82 (d, 2H, CH=CH), 6.51 (s, 3H, 3 × OH), 6.57-7.21 (m, 7H, Ar-CH)

Table no.3. The mitotic indices of Pure Resveratrol and synthesized Resveratrol at various concentrations.

Drug	Mitotic Index Concentration (µg/ml)				F(425)
	25	50	100	200	
Cyclophosphamide	0.79 ± 0.02	0.45 ± 0.01	0.25 ± 0.01	0.10 ± 0.01	109.96 P □ 0.001
Pure Resveratrol	0.84 ± 0.01	0.50 ± 0.01	0.22 ± 0.01	0.10 ± 0.01	414.64 P □ 0.001
Synthesized resveratrol	0.72 ± 0.02	0.50 ± 0.05	0.22 ± 0.003	0.44 ± 0.01	832.09 P □ 0.001

REFERENCES

Alonso E, Ramon DJ and Miguel Y, simple synthesis of 5-Substituted Resorcinols: A revisited family of interesting bioactive molecules, J.Org.Chem., 62, 1997, 417-421.

Aprem H and Handique, Antimutagenic activity of tryptophan and alanine, Current Science, 72, 1997, 578-580.

Hoda O, Badaruddoza and Afzal M, Impact of vitamin B-12 against pesticide induced mitoinhibition and clastogeny, Cytologia, 57, 1992, 353-358.

Jeandet P, Adrian M, Bessis R and Joubert JM, Induction of phytoalexin (Resveratrol) synthesis in grapevine leaves treated with Aluminium chloride, J.Agric.Food Chem., 44, 1996, 1979-1981.