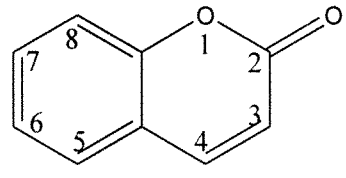
CHAPTER-I

INTRODUCTION

I.1 General Introduction to benzopyran-2H-ones

In 1820 a great scientist Vogel succeeded to isolate 2H-1-benzopyran-2-one from the beans of Tonka tree.[1] 2H-1-Benzopyran-2-one is commonly known as 'Coumarin' which originates from a Caribbean word '*coumarou*' for the tonka tree. Derivatives of 2H-1-benzopyran-2-one are widely distributed in nature either in free state or in combined state.[2]



2H-1-Benzopyran-2-one or Coumarin

Some of the naturally occurring 2H-1-benzopyran-2-ones are *asculatin, fraxetin, daphnetin, ayapin, umbelliferon.* Another interesting group of widely occurring 2H-1-benzopyran-2-ones is furo fused benzopyrones.

Naturally furo fused benzopyrones, are commonly occurring in two types 2H-furo[3,2-*g*]-1-benzopyran-2-one, called psoralen and 2H-furo[2,3-*h*]-1-benzopyran-2-one, called angelicin.

Psoralens and angelicins are present especially in plant species of *Umbelliferae*, *Rutaceae* and *Leguminosae*. Psoralens show marked photosensitizing activities of which the skin photosensitization followed by dark pigmentation is the best known.[3]

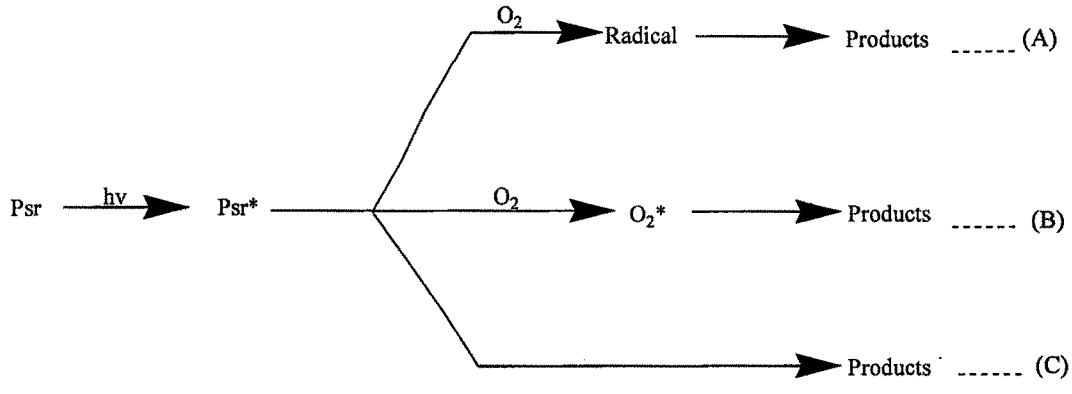
The ancient Hindus, Turks, Egyptians and other Orientals have exploited this property of psoralens in popular medicines by using plants or herbs for the treatment of vitiligo since ancient times.[4] In 1948 El Mofty rationalized psoralen therapy by using the active components of *Ammi majus* (mainly 8-methoxypsoralen **1** and 5-methoxypsoralen **2**), isolated in a chemically pure states.[5] Scientific interest in psoralens has markedly grown over the last few decades after the clinical introduction of 8-methoxypsoralen **1** followed by UV-A irradiation in the treatment of psoriasis by Parrish et al.[6]

The photochemotherapy of psoralen, known as 'PUVA', has been studied for various skin diseases along with other derivatives of furosubstituted 2H-1-benzopyran-2-ones.[7] The new studies are directed towards better knowledge of mechanism of psoralen action and a

more precise evaluation of psoralen toxicity has been developed. Psoralens and angelicins have also been widely studied as photoactive probes of structure and function of nucleic acids.[8]

I.2 General Mechanism of Photosensitization:

Three different pathways may be involved in photosensitization by psoralens and angelicins.[9]



Psr = Psoralen

The pathway (A) and (B) involve oxygen and are referred to as photodynamic pathways while (C) doesn't involve oxygen and it is called anoxic pathway. The pathway (A) involves substrate photooxidation by radicals[10] whereas the pathway (B) involves generation of singlet oxygen by energy transfer. These dynamic pathways are biologically important since they may affect membranes (i.e. oxidation of unsaturated lipids), proteins and enzymes (i.e. oxidation of amino acids) and nucleic acids (as guanosine is susceptible to oxidation). The pathway (C) involves photoreactions between psoralens/angelicins and a substrate not involving oxygen. Thus the psoralens and angelicins seem to be able to interact with living cells at various levels such as receptors involving photo interaction with protein membranes and cytoplasmic moiety including inactivation of enzymes and ribosomes, cell membrane involving lipid peroxidation and 2+2 cycloaddition photoreaction with DNA and chromatins.

The research done in this field has evidenced that the photodamage to the cell membrane constituents, involves both the oxygen dependent photodynamic and photo independent anoxic pathways. The photodynamic pathway mainly leads to lipid peroxidations[11] and formation of cross-linking in the ghost protein[12] while the anoxic pathway leads to photo cycloaddition reaction between the psoralens and unsaturated fatty acids[13-15] (Figure-A).

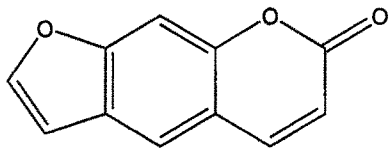
I.3 Effects of psoralens and angelicins as chemotherapeutic agents:

(i) Photoreaction between psoralen and unsaturated fatty acids:

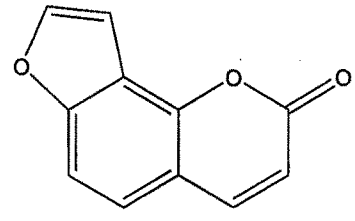
First evidence of photoreaction between psoralen and unsaturated fatty acids using trimethylpsoralen **3** was reported by Kittler and Lober.[13] Specht *et al*[15] studied the photoreaction (360 nm) between trimethylpsoralen **3** and oleic acid methyl ester (OAME) by irradiating a methanolic solution of compound **3.** The resulting photoproducts were separated by reverse phase HPLC. The four products with retention time longer than OAME were detected. On the basis of mass, NMR, UV absorption spectra and fluorescence properties, the photocompounds were shown to be cyclobutane adducts, derived from [2+2] photocycloaddition reactions, in which the pyrane-side double bond of psoralen was involved.

Caffieri *et al* studied the photoreactions between psoralens and methyl ester of linolenic acid **4** (LAME) .[14] The photoadduct between psoralen and linolenic ester was isolated by reverse phase HPLC. When labeled psoralen was used, the isolated photoadducts proved to be radioactive confirming the presence of the psoralen moiety. Moreover, when photoadduct 'psoralen-linolenic ester' (Figure-A) was irradiated at 254 nm, it underwent photoreversion forming psoralen and linolenic ester. On the basis of its spectroscopic properties and ability to undergo photoreversion, the authors suggested that the photoadducts is derived from a 2+2 cycloaddition between double bond of the fatty acid and pyrane-side double bond of psoralens. It is found that only one psoralen molecule is linked per fatty acid molecule. The region- and stereo-arrangement of the two moieties with respect to

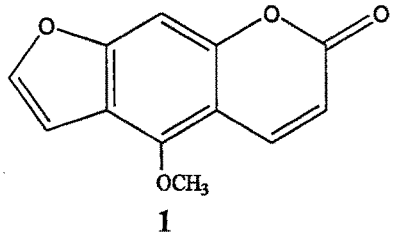
cyclobutane is also confirmed, the two remaining parts of the linolenic acid are in trans geometry with respect to the cyclobutane ring.[16-17] Similarly photoreaction between angelicins with methyl ester of linolenic acid (LAME) showed a 2+2 cycloaddition reaction taking place between the pyrone-side double bond of angelicin and central double bond of LAME.[18] The photoadduct isolated showed the same molecular structure as psoralen did.



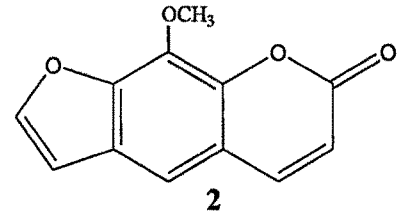
Psoralen



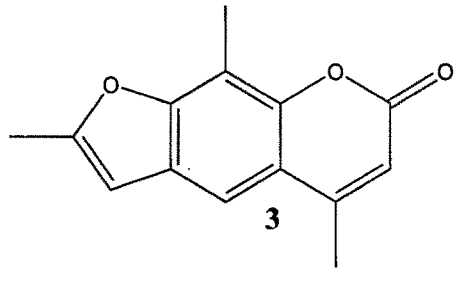
Angelicin



1



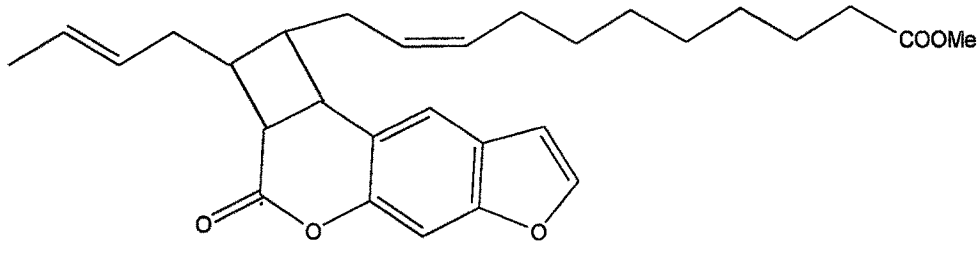
2



3

$CH_3CH_2HC{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOMe$

4



Photoadduct psoralen- linolenic ester

Figure-A

Experiment of photoreactions between angelicins and unsaturated fatty acids was also carried out *in vivo*. In this experiment, ^{3}H-Trimethyl angelicin (^{3}H-TMA) **5**, was applied on the shaven skin of Wistar rats, the skin was irradiated (I=20 W/ml) after 15 min incubation then epidermis was separated from the dermis and subjected to dialysis under sink conditions to remove unbound ^{3}H-TMA. Selective extraction of the epidermis produced three phases viz. the lipid, DNA/RNA and the protein. The lipid phase was subjected to methanolysis to transform the natural unsaturated fatty acids and the photoadducts in the corresponding methyl esters. The transesterified lipid phase was examined by HPLC. In the chromatogram other than linolenic acid and oleic acid methyl esters, some peaks having a retention time strictly similar to those shown by the photoadducts prepared *in vitro* were also present indicating that the photocycloaddition between angelicins and unsaturated fatty acids not only takes place *in vitro* but also *in vivo*.[19]

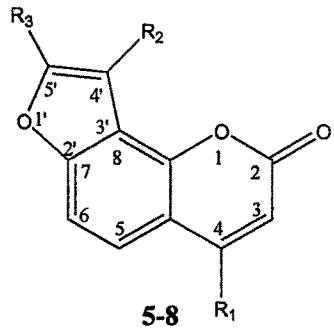
(ii) Photohaemolysis of erythrocytes by psoralens and angelicins:

In 1986 Potapenko studied modification of erythrocytes membrane induced by psoralen which showed photohaemolysis in red blood cells under special condition of incubation at 37°C.[20] Vedaldi *et al* observed that psoralens and 3-carboethoxy psoralens were most active while other derivatives of psoralens showed poor or no activity.[21] They observed that there is an increase in membrane permeability to cations in terms of potassium leakage. A marked increase in potassium release is observed when erythrocytes are irradiated in presence of psoralens. This effect is partly concentration dependent and is observed without any thermal incubation, supporting that the psoralen is able to photoinduce lesions to erythrocyte membrane. This toxic effect has also been studied *in vivo* by topical application of psoralens to the skin of *Albino guinea pigs* showing the damage induced in circulating red blood cells.[22] Results obtained showed that topical PUVA, on *guineapig* skin, induces erythrocyte damage in terms of increased osmotic fragility, partial haemolysis and lipid peroxidations. This toxic effect therefore should be taken into account when topical photochemotherapy with the psoralens is used.

Post Irradiation Dark Haemolysis:

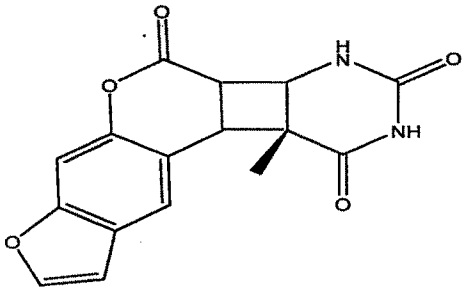
When a suspention of erythrocyte was added to a pre-irradiated ethanolic solution of psoralen, there was observed evident haemolysis, after thermal incubation of the red blood cells. This effect has been defined as Post Irradiation Dark Haemolysis (PIDH).

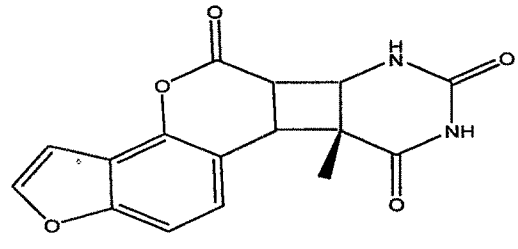
In ethanol or some organic solvent, PUVA and PIDH are found to be very strong and is also present at the lower concentration of psoralens (1.4×10^{-5} M). While in water and saline solution PIDH was found to be very poor. Angelicins **5-10,** showed marked photobiological activity which was observed on *E.Coli* culture, *Ehrlich Ascites* tumor cells and on T_2 *Phase* virus. In this connection a good correlation has been observed between the rate constant of the photoreactions and the photobiological activities.



5-8

R_1	R_2	R_3	Compound no.
CH_3	CH_3	CH_3	**5**
CH_3	CH_2OH	CH_3	**6**
CH_3	CH_2OCH_3	CH_3	**7**
CH_3	CH_2NH_2:HCl	CH_3	**8**





Complexes of psoralen and angelicin with thiamine residue of DNA

A preliminary complex in the ground state occurs between psoralens/angelicins and the duplex DNA and this molecular complex play the most important role in the successive cycloaddition.[23-25]

It is found that introduction of a cationic group in the 4' position in angelicin (compound **8**), stongly increases the binding parameters of the complex formed with DNA.

This interaction is analogous to that of some anti tumor or antibiotics, such as *Daunomycin, Adriamycin, Actinomycin* etc. This provoked the increased affinity of the cationic compounds **8** towards DNA in comparison to the parent compound angelicin.

When a hydroxymethyl group is introduced into the 4' position of the angelicin (i.e. compound **6**), its affinity for the DNA complex formation in dark was slightly lowered in comparison with the parent angelicin. When methoxy methyl group is introduced into the 4' position (i.e. compound **7**), the extent of complexation is markedly decreased.

From all these data, the following generalization can be made. The studies of psoralens and angelicins show that, in the photoreactions, DNA form only monofunctional adduct with angelicins and can not form interstrand cross-linkage in native DNA, thus show only monofunctional lesion. But psoralens show bifunctional lesions which was further supported by the fluorescent data of angelicins derivatives.

Flow dichroism measurements as well as fluorescence quenching data strongly support the fact that the psoralens and angelicins, when complexed with DNA, undergo intercalation between two pairs of the macromolecules.

Angelicins showed low activity compared to psoralens but at the same time angelicins showed no skin phototoxicity. While on account of their bifunctional lesions, linearly psoralens showed skin phototoxicity.

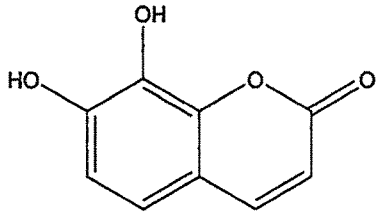
Angelicins have shown evident photobiological activity both on *Ehrlich ascites* tumor cells, where DNA and RNA synthesis was strongly inhibited and on T_2 *Phase* virus, where their inactivity was markedly affected.

The data have shown that a close correlation exists between photobinding capacity to DNA and photobiological activity. In case of angelicins this correlation is direct evidence of monofunctional photodamage as well as inability to induce skin toxicity. While for psoralens, the bifunctional lesions and skin toxicity can be correlated.

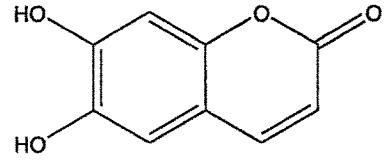
Thus the knowledge of the mechanism of action of substituted 2H-1-benzopyran-2-ones at the molecular level allows a better understanding of their activity and possible application or valid help in the drug design of new substituted 2H-1-benzopyran-2-ones with reduced toxicity.

Many naturally occurring 2H-1-benzopyran-2-ones are also found to affect the living cells of plants and animals in various ways. Biochemical properties of the natural 2H-1-benzopyran-2-ones have been reviewed by Bose[26]. The effect of *Asculetin* and *daphnetin* on seed germination was studied by Sigmund.[27] He noted that the 2H-1-benzopyran-2-ones themselves inhibit the germination and subsequent root growth of the plants. Various

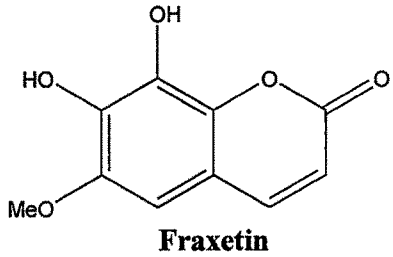
substituted 2H-1-benzopyran-2-ones have been found to show important biological activities and following is the list of such derivatives with their activities.



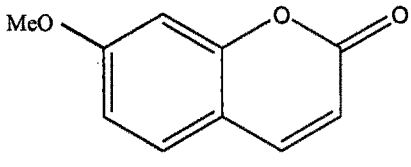
Daphnetin



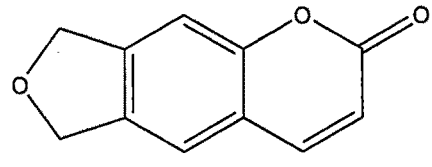
Aesculatin



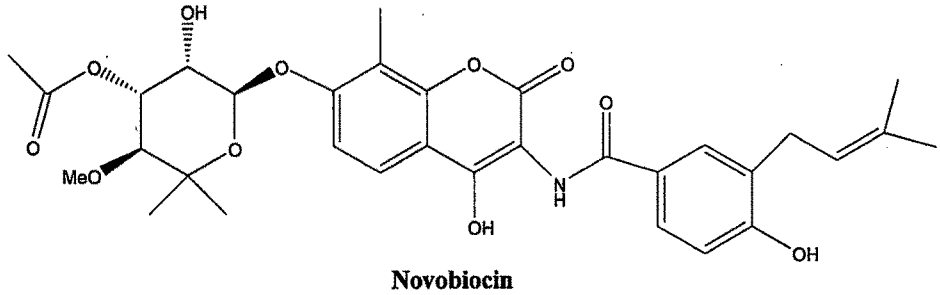
Fraxetin



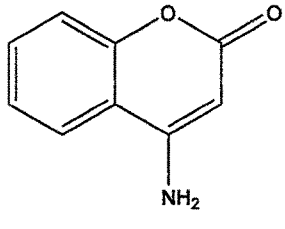
Herniarin



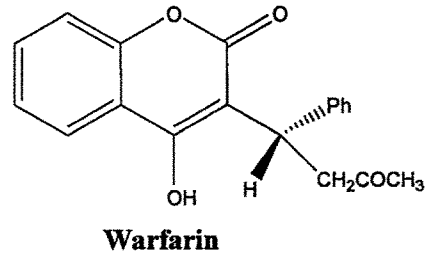
Ayapin



Novobiocin



4-Amino coumarin



Warfarin

Coumarin: Acts as narcotic for some animals, as a sedative and hypnotic for mice.[28]

Fraxetin: Causes paralysis of the central nervous system of frogs and mice on intravenous injection.[29] It is also found to be superior to atophan in the treatment of gout.[30]

Ayapin and Herniarin: They show haemostatic properties and are active in vitro as well as in vivo.[31]

Novobiocin: It is an antibiotic isolated from Streptomyces species and its antibacterial spectrum is comparable with the penicillin and the erythromycin. However, in vitro it is very less active.[32]

4-Amino coumarin: It increases activity of central nervous system.[33]

Warfarin: It is a useful anticoagulant drug.

I.4 General spectral properties of 2H-1-benzopyran-2-ones:

UV spectra:

UV absorption spectra can distinguish between 2H-1-benzopyran-2-ones with other isomeric forms. Normally 2H-1-benzopyran-4-ones have a strong absorption at 240-250 nm, whereas 2H-1-benzopyran-2-ones have a weak absorption at 240-250 nm. The alkyl substituted 2H-1-benzopyran-2-ones shows absorption at 271-278 nm and 311-318 nm due to the benzene and pyrone ring respectively.[34] In coumarin substitution at C-3 shows a small change in absorption, leading to a small hypsochromic shift in 311 nm maxima while the substitution at C-5, C-7 and C-8 show a bathochromic shift in 271 nm maxima.

IR spectra:

Various functional groups can be revealed from IR spectra of the 2H-1-benzopyran-2-ones. These compounds show carbonyl stretching frequency in 1700-1750 cm^{-1} range, whereas 2H-1-benzopyran-4-ones show in the range of 1650 cm^{-1} which can be clearly distinguished from its isomer.

^{1}HNMR spectra:

^{1}H-NMR is the most powerful tool for confirming the structural properties of any organic compound and indeed, 2H-1-benzopyran-2-ones can be distinguished from its closely related isomeric products. Steck and Mazurek[35] efficiently explained spectra-structure correlation rules with an outstanding interpretation.

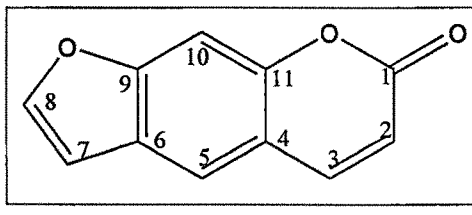
Signals at δ 6.11 – 6.14, with a pair of doublets, J=9.5 Hz and δ 7.51-8.31, J=9.5 Hz in proton NMR have revealed unsubstituted 2H-1-benzopyran-2-one (coumarin) ring system, showing the presence of protons at C-3 and C-4 respectively. On the other hand, C-8 proton was shown to be further downfield in the range of δ 8.00-9.00. The measurement of Nuclear Overhouser Effect (nOe) also provides considerable support in assigning the geometry of the 2H-1-benzopyran-2-ones, especially in substituted derivatives.

Linearly fused pyran ring showed higher values of δ in ^{1}HNMR spectra while angularly fused pyran ring showed lower values of δ in ^{1}HNMR spectra.[36]

^{13}CNMR spectra:

Fourier-transformed combinations and advancement in computer technology have made the NMR a powerful and sensitive tool for structural elucidation of complex organic compounds. ^{13}CNMR provides correct information about different types of (i.e. non-equivalent) carbon atoms present in the given compounds. Many publications have appeared assigning ^{13}C chemical shifts and extensive assignments of carbon-proton coupling presented for the hydroxyl and methoxy substituted 2H-1-benzopyran-2-ones[37] as well for furosubstituted 2H-1-benzopyran-2-ones.[38]

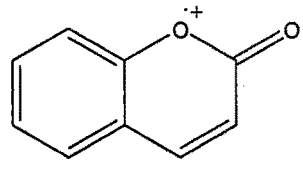
Following is the list of general chemical shifts for carbon in ^{13}CNMR for given compound.



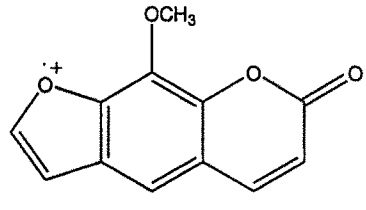
C-1 = 160.4	C-2 = 116.5	C-3 = 118.8
C-4 = 117.8	C-5 = 124.4	C-6 = 117.2
C-7 = 116.4	C-8 = 131.8	C-9 = 153.9
C-10 = 128.1	C-11 = 143.6	

Mass spectroscopy:

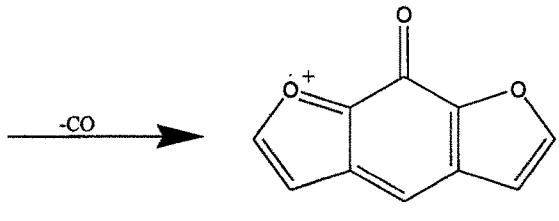
2H-1-Benzopyran-2-ones show intense molecular ion peak, showing very stable heterocyclic ring system fragment formation. A strong molecular ion peak, m/z =146 (76%) and a base peak, m/z = 118 (100%) were observed. This occurs through the loss of carbon monoxide from the pyran ring[39] resulting in the formation of molecular ion, benzofuran. The loss of carbon monoxide from methoxy furanobenzopyron showed molecular ion peak, m/z = 173 (50%). It is observed that the presence of furan ring in 2H-1-benzopyran-2-ones, normally, does not alter the fragmentation.



m/z = 146, 76% **m/z = 118, 100%**



m/z = 216, 100% **m/z = 201, 22%**

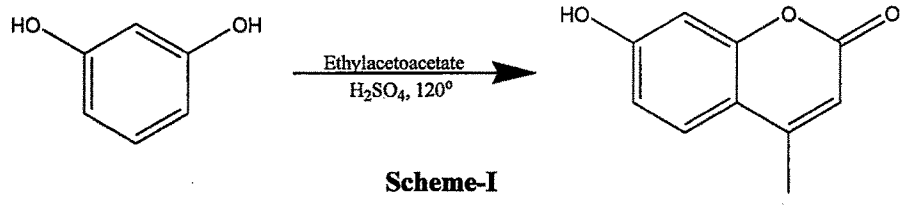


m/z = 173, 56%

I.5 General approaches applied for synthesis of 2H-1-benzopyran-2-ones

Pechmann condensation has been utilised in the formation of 2H-1-benzopyran-2-one involving the condensation of phenols with a-keto esters in presence of condensing reagents such as H_2SO_4, P_2O_5, $POCl_3$ and $AlCl_3$[40-42](Scheme-I).

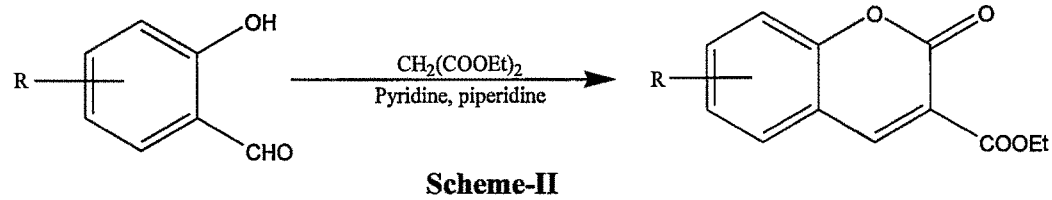
Pechman synthesis



Scheme-I

Synthesis of substituted 2H-1-benzopyran-2-ones has been reported via Knoevenagel condensation [43] (Scheme-II).

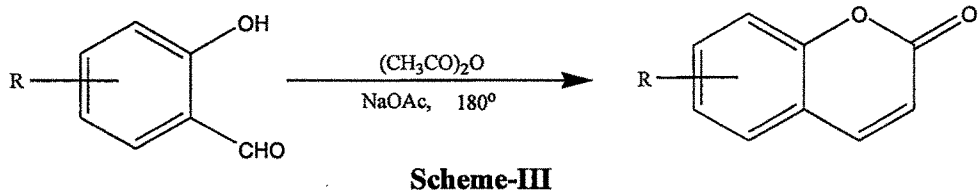
Knoevenagel condensation



Scheme-II

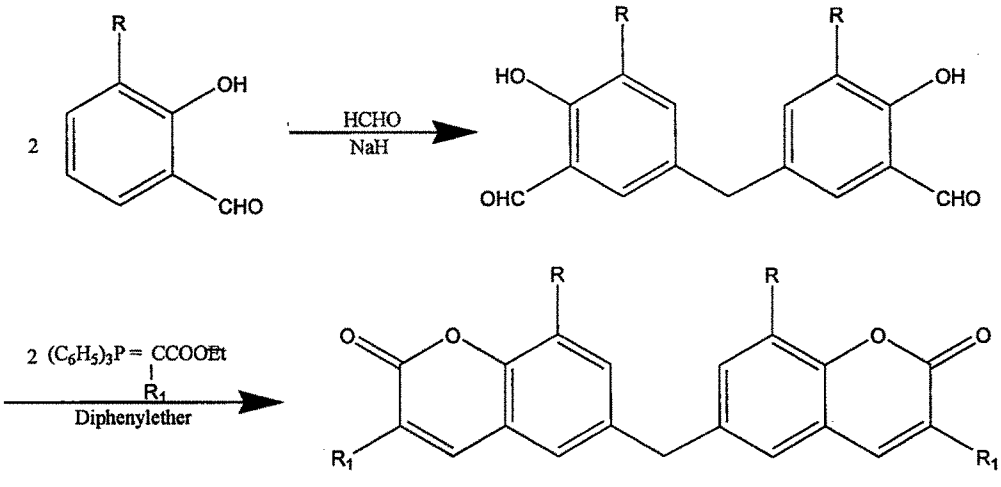
In another synthesis 2H-1-Benzopyran-2-ones have also been prepared using Perkin condensation[44,45] (Scheme-III).

Perkin synthesis

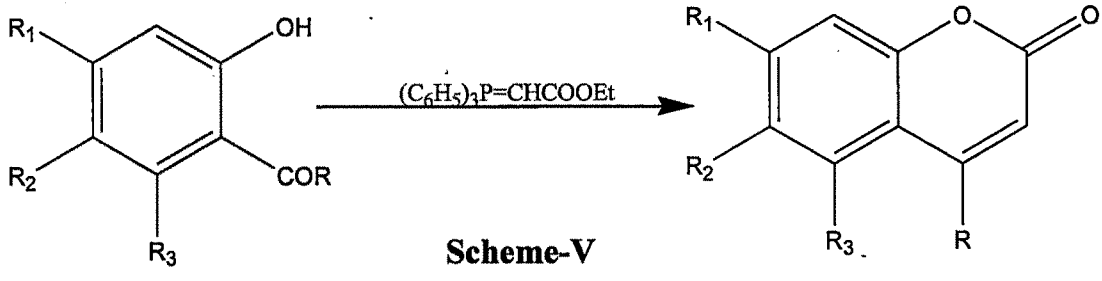


Scheme-III

Recently, Brahmbhatt *et al* prepared 2H-1-dibenzopyran-2-ones using strategy of Wittig[46] (Scheme-IV) and Mali *et al* utilized Wittig reaction for synthesis of 2H-1-benzopyran-2-ones[47] (Scheme-V).

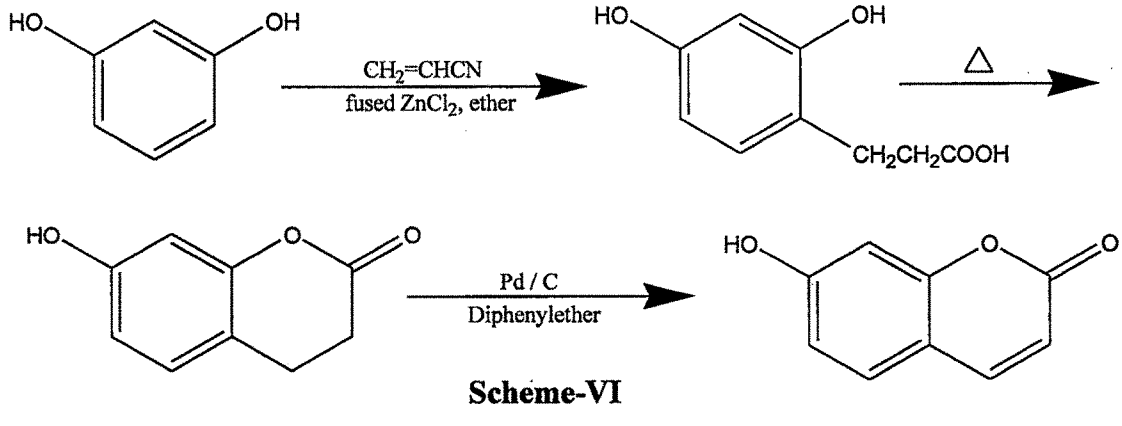


Scheme-IV



Scheme-V

In yet another approach, Chatterjee *et al*[48] have synthesized 2H-1-benzopyran-2-one using acrylonitrile. The key step involves Pd/C catalysed dehydrogenation (Scheme-VI).



Scheme-VI

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