www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 1097-1101, April-June 2010

Synthesis, Characterization & Antibacterial Activity of 7, 4^1 – Dihydroxy, 3^1-Methoxy Flavones

Murthy Y L N[1]* , I V Kasi viswanath[2]† ,E.Nageswar pandit[1]

[1]Dept of Organic Chemistry, Andhra University, Visakhapatnam,India

[2] Dept of Organic Chemistry, Dr B R Ambedkar University,Srikakulam,India

**Corres.author : murthyyln@yahoo.co.in*
† part of the Ph.D thesis investigations of I V kasi viswanath[2]

ABSTRACT: The Synthesis of 7,4^1- dihydroxy,3^1-methoxy flavones is carried out and the synthesized compounds are characterized by IR, 1^HNMR data. The antibacterial activity of the above compound is investigated and the data is presented. .Inhibition zones (MIC- mm's) were calculated and presented. The results are encouraging

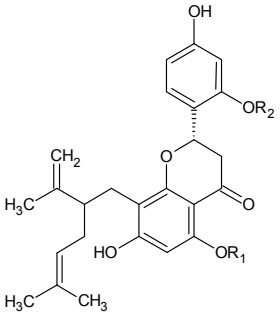
KEY WORDS : 7,4^1- dihydroxy,3^1-methoxy flavones , synthesis, antibacterial activity, inhibition zones.

INTRODUCTION

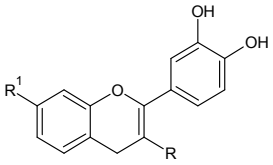
Flavanoids are a major class of oxygen containing hetrocyclic natural products and are wide spread in green plants.[1]The major group of plant poly phenols is represented by flavanoids and recent review has estimated their number as 6500 in the plant kingdom.[2] The major flavanoids in the plant extracts is quercetin, followed by myricetin and kaempferol. Chrysin is a flavones widely distributed in plants which was reported to have many biological Activities including antibacterial,[3] antioxidant,[4] antiinflamatory,[5] antiallergic,[6] anticancer,[7] antiestrogenic [8] activities. Further more, Chrysin is also found to have tyrosinase inhibitory activity,[9] moderate aromatase inhibitory activity.[10,11,12] In human cell culture studies, quercetin has been shown to inhibit histamine release. They have been many bioassay guided searches for cytotoxic antitumor agents in plants especially they are known to be used in folk medicine for this purpose. This has led to the isolation and identification of quite a large number of active constituents from all the different flavanoid classes. Two new lavandulyted flavanones,(2S) -2^1-methoxy kurarinone(1) and (-) kurarinone(2), were isolated from the root of sophora flavescens, together with two known larvandulyl flavanones, sophora flavanone G(II) and leachianone A(12). All these compounds 1 to 4 showed significant cytotoxicities with IC_{50} values of 13.7,18.5,12.5 and 11.3 µm against human myeloid leukemia HL-60 cells.[13]A series of 3,7-disubstituted 2-(3^1,4^1- dihydroxy phenyl) flavones(5-8)was synthesized by Frederique A. A. van acker et.al.[14] All the compounds are potent radical scavengers, potential caridoprotectors and showed antioxidant activity.

J.M. Rao et.al[15] reported the synthesis of three series of analogous of chrysin(9-11) and their antibacterial activities. Flavaone acetic acid and its related compounds were found to exhibit antitumor activity.[16] 6-methyl derivatives of flvaone 8-acetic acid (12) also found to posses anti tumor activity.[17]

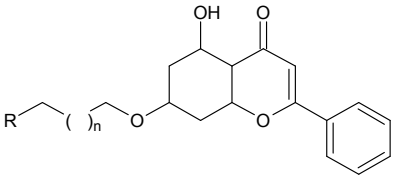
Chemistry : The synthesis of 7,4^1-dihydoxy,3^1-methoxy flavones is presented in the following sequence (Scheme I) and characterized by I.R, ^{1}H NMR data. The antibacterial activity of Compound (VI) is investigated and the data is presented in table I , inhibition zone values (mms) were calculated and presented in tabular form and in the form of diagram(fig 1.)



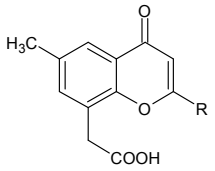
1 $R_1=R_2=CH_3$
2 $R_1=CH_3$, $R_2=H$
3 $R_1=R_2=H$
4 $R_1=H$, $R_2=CH_3$



5 R= H R^1= OH
6 R= H R^1= OCH_3
7 R= H R^1= OCH_2CH_2OH
8 R= H R^1= $O(CH_2)_3N(CH_3)_2$



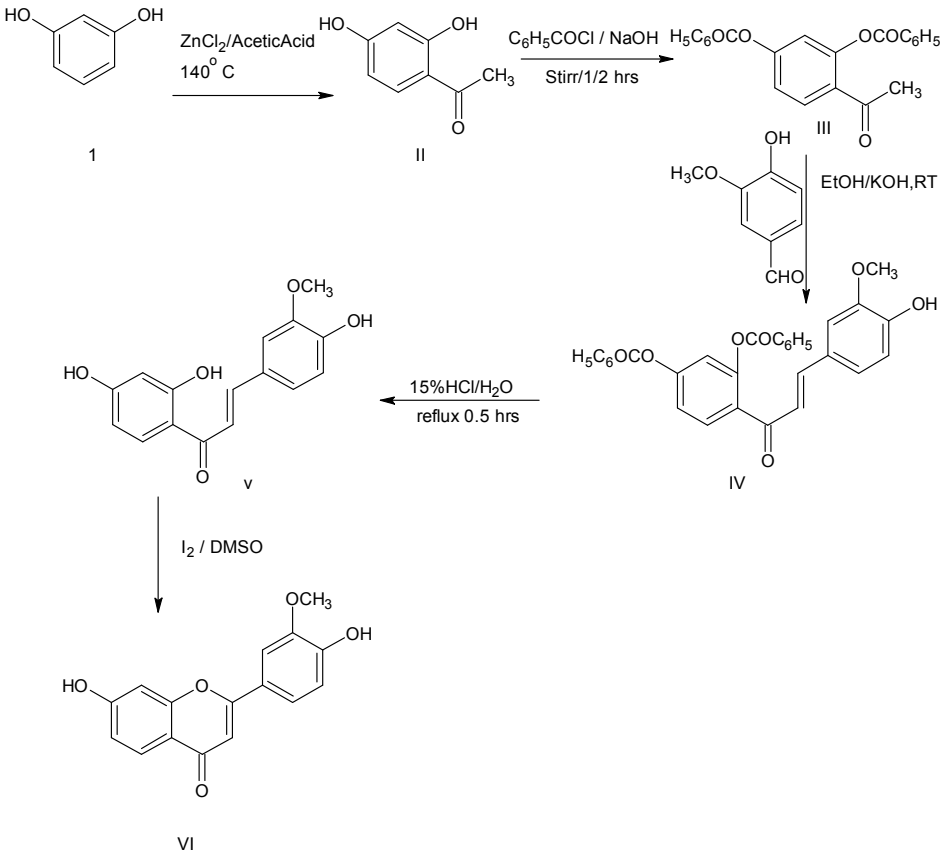
n =1, 2, 4
(9a-17d) (10a-10d) (11a-11d)
9a-11a R=Morpholine
9b-11b R=N-methyl piperizine
9c-11c R= piperizine
9d-11d R=N, N-methyl amine



12

R = Aromatic

Scheme I



EXPERIMENTAL DETAILS

Preparation of Resacetophenone (II):

Freshly fused 33 gm of $ZnCl_2$ was dissolved in 32ml of acetic acid while heating and when all the $ZnCl_2$ is almost dissolved, 22 g of resorcinol was added and heated to 140-150^{0}C for 15 min with stirring. After that this is left 1 hr and then 100 ml of 1:1 HCl was added to break the zinc chloride complex, Within 5 minutes precipitation commenced when the mixture came to room temperature. It is cooled to 5^{0}C and then filtered. The precipitate was crystallized from 20% HCl to give resacetophenone needles.

m.p.→ 144^{0}C
TLC: R_F : 0.4 (Hexane: etoac, 8:2)
Molecular formula: $C_8H_8O_3$
I.R. (υ_{max} ^{nujuol}): 3309 cm$^{-1,}$ (-OH Str); 1680 cm^{-1} (C=O Str)
1^H NMR ($CDCl_3$ /TMS): δ2.45 (S), 6.3(S), 6.8(d), 11.45 (S, 2-OH)

Benzoylation of Resacetophenone(III):

To anhydrous resacetophenone (1 mol), benzoyl chloride (1 mol) and NaOH solutions are added; the contents were stirred for half an hour in ice bath. Then the reaction mixture is poured in ice-coldwater. Dark green solid is formed which is filtered and crystallized from methanol to get the crystals of dibenzoate of resacetophenone in 60% yield, m.p. – 80^{0}C.

Condensation of the dibenzoate of resacetophenone with 4 –hydroxy 3-methoxy benzaldehyde (Vanilin):

A mixture of dibenzoate of resacetophenone (0.01 mol) and 4-hydroxy, 3-methoxy benzaldehyde in ethanol (30ml) and aqueous potassium hydroxide (15 gms in 15ml of water) was kept at room temperature for 72 hours. This was neutralized with 1:1 cold HCl in cold conditions and extracted with ether. The ethereal layer was dried over anhydrous Na_2SO_4 and evaporation of the solvent gave snuff coloured product which was recrystallized from methanol,
TLC: R_F : 0.6 (Etoac : $CHCl_3$ 1:9) Yield: 75%.

Hydrolysis of the dibenzoate of the chalcone:

The dibenzoate of the chalcone was refluxed with 50ml of 15% HCl solution for half an hour to hydrolyse the ester groups. Then the contents are poured in ice cold water and extracted with Etoac (50ml). The ethyl acetate layer was dried over anhydrous $MgSO_4$ and the solvent was stripped off by distillation. The crude product was chromatographed over silica gel with n-hexane and ethyl acetate (95:5) as effluent. Further the product was recrystalised from methanol, which yielded yellow coloured crystals. It gave red colouration with $SbCl_3/CCl_4$: a +ve test for chalcones.
m.p. → 139^{0}C
TLC: R_F : → 0.75 (Etoac: $CHCl_3$ 1:9)
1^H NMR ($CDC1_3$/TMS): δ4.0 (-OCH_3,S), δ7.0 – 6.5 (dd, 2 H, olefinic protons), δ7.2 – 7.5, 7.8 (m, 5H, aromatic protons), 9.9, 12.5 (br, s, 3H, phenolic protons).

Synthesis of 7, 4^1 – dihydroxy, 3^1-methoxy flavones(VI):

Compound (V) (0.01mol) was taken in R.B. Flask, 10 ml of dimethyl sulphoxide and Iodine (500 mg) was added to the round bottom flask. This was refluxed on an oil bath at 140-150^0 for one hour. After Cooling, the reaction mixture was poured into ice water and 20 ml of saturated hypo-solution was added and left-over night. The solid was washed with water and chromatographed over a silica gel column. Elution of the column with hexane: etoac (80:20) yielded 7, 4^1-dihydroxy, 3^1-methoxy flavone (VI) and was recrystalized from ethanol. It gave red colouration with Mg/HCl, a +ve test for flavanoids,
m.p. – 144^0.
TLC: R_F : → 0.40 (Etoac:$CHCI_3$, 1:9)
1^H NMR: 4.0 (-OCH_3, 3H, 3), 6.5 (S, 1H), 7.4-76 (m, aromatic protons), 7.8 – 7.9 (m, Aomatic Protons), δ 9.9 (br, s, 2H).

Antibacterial activity studies of 7^1, 4^1 -dihydroxy, 3^1-methoxy Flavone(VI):

The compound (VI) was tested for antibacterial activity against gram positive bacteria: viz, staphylococcus auress, bacillius subtills, Streptococcus griseus and gram – negative bacteria: Eschericahia coli, psuedomonas Aureagionsa at concentrations of 50, 100, 150, 200 µg /ml. The cultures of organisms grown overnight at 37^{0}C, were used for testing the antibacterial activity which was checked employing cup plate method. Nutrient agar medium (Himedia, India) was dissolved in water and p^H was adjusted to 7.0. This was then distributed in 20 ml quantity in boiling tubes; they were then plugged tightly with non-absorbent cotton and sterilized in an autoclave. The bacterial culture (50 µl) was then added aseptically to the agar medium maintained at 45^{0}C, mixed well and poured immediately in sterilized petriplates. Test solutions of different concentrations of compounds (VI) were prepared in DMSO. After hardening, cups of 8 mm diameter each were cut into agar and 50 µl test solutions of varying concentration (50, 100, 150 and 200 µg/ml) were placed in these cups. The plates were incubated at 37^{0}C for 24 hours and the diameter of inhibition zone was measured in mm's. Solvent DMSO was kept as control, which did not have any inhibition zone. The activity was

compared with standard antibiotic amphicillin. (manufactured by Alemdric Ltd., Vadodara, India), antibacterial activities inhibition zones of the compounds are presented in table 1 ,and comparison of inhibition zones in the form of diagram was presented in fig 1.

Table I: Antibacterial activity inhibition zones

Bacteria name	Amphicillin	Compound VI Concentration (µg/ml)			
Gram Positive bacteria		50	100	150	200
Staphylococcus aureus	20	----	13	14	16
Bacillus subtillis	17	11	12	13	15
Streptococcus griseus	18	----	11	11	12
Gram negative					
Escherichia coli	22	12	13	14	16
Psuedomonas aeruginosa	20	----	11	13	14

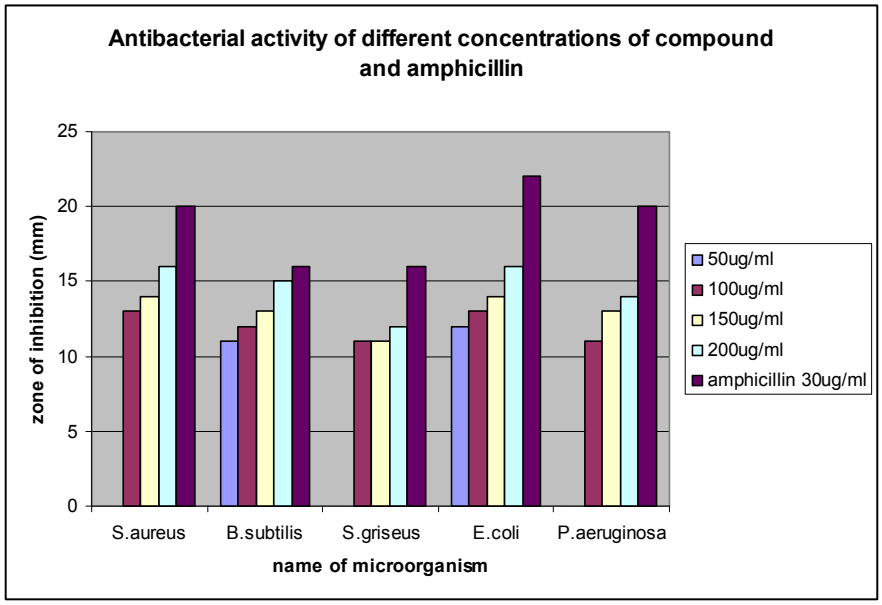


Fig 1

RESULTS AND DISCUSSIONS

Compound VI was confirmed by spectral data(IR,NMR). The antibacterial activity was studied against the five organisms & the minimum inhibition concentrations (MIC) at 50 µg/ml has been observed at 11 for Bacillius subtillis, which shows potential activity, Where as in other strains it was not observed. Flavone (VI) almost equally active as amphicillin at 200 µg/ml level against saphylococus aurers. In case of gram –ve bacteria E-coli: MIC at 50 µg/ml compound IV exhibited MIC : 12 where, it is active at higher concentrations. These studies confirmed that, flavanoids are biologically active at higher concentrations.

ACKNOWLEDGEMENTS

The authors thankful to DRDO authorities for the financial support and also express their gratefulness for Botany department, Andhra University Visakhapatnam, for supplying micro organisms.

REFERENCES

1. Bohm B.A., Introduction to Flavanoids; Gardon & Breach; Amsterdam & Netherlands, 1998.The handbook of Natural Flavanoids; Harborne J.B., Baxter. H., Etd: willey; ehic Chester, U.K. 1999; Vols 1& 2.

2. Harborne, J.B., Williamson C.A, Advances in flavonoid research since 1992, Phytochemistry, 2000, 55, 481.

3. Gais N., Rahman M.M., Rashid M.A. Kashino H., Nageswara K., Nakata.T., Fitoterapia, 1996, 67 (6), 554.

4. Hecker M., Presis C., Klemm P., Busser., Inhibition by antioxidants of nitric oxide synthase expression in murine macrophages: role of nuclear factor kappa B and interferon regulatory factor 1.,Br. J. Pharmacol., 1996, 118, 2178.

5. Fishkin R.J., Winslow J.T., Endotoxin-induced reduction of social investigation by mice: interaction with amphetamine and anti-inflammatory drugs, Psychopharmacology (Berl.) 1997,132, 335.

6. Pearce F.L., Befs, A.D., Blenenstock J., Mucosal mast cells [*1], [*2]: III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells **,**J.allegy Clin. Immunolgy, 1984, 73, 819.

7. Habtemariam S., Flavonoids As Inhibitors or Enhancers of the Cytotoxicity of Tumor Necrosis Factor-α in L-929 Tumor Cells, J..Nat. Products., 1997, 60, 775.

8. Kao Y.C., Zhou C., Sherman M., Loughton C.A., Chen S**.,** Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: A site- directed mutagenesis study. Environ. Health Perspect., 1998, 106, 85.

9. Kubo, I., Kinst – Hori I., Choudari S.K., Kubo Y., Sanchez Y., Ogura T., Flavanols from Heterotheea inuloides : Tyrosinase Inhibitory Activity and structural criteria, Bioorg. Med. Chem.., 2000, 8, 1749-55.

10. Suresh C.T., Leena S., Sari M., Risto S., Inhibition of 17β-Estradiol Formation by Isoflavonoids and Flavonoids in Cultured JEG-3 Cells: Search for Aromatase-Targeting Dietary Compounds, J. Med. Food., 1999, 2, 235.

11. Bors W., Heller W., Michel C., Stettmaier K., Handbook of Antioxidants, marcel – Dekker, New York, 1996, 409.

12. Flavanoids in health and disease (Rice – Evans, C.A. and Packer L.Ed.,), Marcel –Dekker, New York, 1998.

13. Tai – Hyun kang, Sei-joen Jeong, Won Gilko, Cytotoxic Lavandulyl Flavanones from *Sophora flavescens* J. Nat. Prod., 2000, 63, 680-681.

14. Frederique A.A. Van Acker., Jos. A. Hageman, Guido R.M.M. Haenen., M.P.B. Menge., Synthesis of Novel 3,7-Substituted-2-(3‘,4‘-dihydroxyphenyl)flavones with Improved Antioxidant Activity J. Med. Chem., 2000, 43, 3752-3760.

15. K. Suresh Babu, T. Hari Babu, P.V. Srinivas, K. Hari Kishore, V.S.N. Murthy, and J.Madhusudhan Rao., Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agents, Bioorganic & medicinal chemistry Letters, 2006, 16, 221-224.

16. Fang Jian – Yun, thomson Colin., Hydrogen-bonding effects, electrostatic potential, and the antitumor activity of flavone acetic acid and related compounds. I. Ab initio studies on the first stable conformations, Int-J.Quantum-chem., 1995, 54 (5), 313-24 .

17. Aitkern, R. Alan, Bibby, Michael C., Double, John Philips, Roger.M., sharma, Sivakumar, Synthesis and anti-tumour activity of 6-methyl derivatives of flavone-8-acetic acid (FAA) Bio org. med. Chem. Lett., 1994, 4 (19), 2313 – 16 .

*****