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Efficient Synthesis of 3-Methyl-Flavanones and Evaluation of Their Anti-Bacterial Activity

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A series of 2-phenyl-2,3-dihydrochromon-4-one derivatives (flavanone derivatives) were synthesized by silica gel assisted isomerization of several α-methyl-2'-hydroxy chalcones in 74%—88% yield. These flavanones were further oxidized to 3-methyl flavones by using iodine in dimethyl sulphoxide at 60 ℃ in presence of acid. The newly synthesized derivatives were evaluated for *in vitro* study against *Staphylococcus aureus*, *Micrococcus luteus* and *Staphylococcus epidermis*.

Keywords cyclization, oxidation, chalcones, silica gel, 3-methyl-flavanones

Introduction

Flavonoids are polyphenolic compounds that are widely distributed in plants and have attracted considerable attention due to their numerous pharmacological applications. Flavonoids are occurring in nature in free state or as glycosides associated with tannins (flavanoids). The derivatives of phenol present as glycosidal form and chemically they are derivatives of chromone ring.[1,2] Natural and synthetic flavanoids have attracted considerable attention because of their interesting biological activities, including anti-oxidant, antifungal, anti-bacterial, anti-inflammatory, antitumor, anti-asthmatic, antiviral, antihypertensive, estrogenic, antixiolytic, diuretic activity and inhibition of hormone-dependent proliferation of cancer cells.[3-7] Flavanoids inhibit xanthine-oxidase enzyme and have superoxide-scavenging activities. Therefore they could be a promising remedy for human gout and ischemia by decreasing both uric acid and superoxide concentration in human tissues.

The reaction of flavanone synthesis can be performed using acids,[8] bases,[9,10] light,[11] heat,[12] microwave[13] or electrons.[14] There are numerous methods available for the synthesis of flavones like Kostanecki,[15] Allan Robinson,[16] Mahal-Venkataraman,[17] oxidation of 2'-hydroxychalcone, the Wheeler method and others. Many of these methods for synthesis of flavanones and flavones required more amount of heating during the reaction and the yields of the products obtained were less (50%—60%).[18] The reaction of 2'-hydroxychalcones with palladium(II) acetate, Co (salpr) under oxygen atmosphere and potassium ferricyanide using phase transfer catalysts[19] leads to the formation of flavanones. Yields of these reactions were low to moderate. Various solid catalysts have been applied for flavonoid synthesis, such as magnesium oxide,[20] zinc, alumina, barium hydroxides, hydrotalcites and natural phosphates modified with $NaNO_3$ or KF. However, most of them require the use of expensive toxic solvents to facilitate the heat and mass transfer in the liquid phase reaction systems.[21]

The acid catalyzed cyclization can be carried out by refluxing the chalcone in acetic acid, in the presence of acid catalyst such as H_2SO_4 or H_3PO_4[22] and ethanol or other suitable solvents. Basic conditions are seldomly used due to decomposition or retroaldol reaction. Although 2'-hydroxychalcones may also be cyclized to flavanones with acidic (H_2SO_4 in methanol, CH_3COOH and phosphoric acid), basic (pyridine and DBU) reagents and microwave irradiation, most of the employed methods require prolonged reaction time at high temperature.

Alternatively flavanones can be prepared from the oxidative cyclization of cinnamic acids and phenols with phosphoric acid or 2'-hydroxyacetophenones and benzaldehydes with piperdine in one step. But the yields of the former are very low and the latter are not applicable for flavanones.

Synthesis of flavones is reported using heterogeneous catalyst molecular iodine loaded neutral alumina under microwave irradiations.[23] CuI catalyzed cascade oxa-Michael-oxidation, using chalcones as substrates, mediated by the ionic liquid [bmim][NTf_2] also gives flavones.[24] But this method takes more time even at high temperature. Menezes *et. al*.[25] followed the strategy of using chalcones for the synthesis of flavones. I_2/DMSO/H_2SO_4 system was used for the synthesis of

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bromo-substituted flavone-like troponoid compounds.[26] Earlier use of iodine has been reported for dehydrogenation of dihydropyridazin-3(2*H*)-one, deallylation of allyl-aryl ethers, oxidation of *β*-anilino-dihydrochalcones to *β*-anilino-chalcones.[27] In this paper, we wish to report silica gel assisted isomerization of several *α*-methyl 2'-hydroxychalcones yielding corresponding flavanones. These flavanones are converted to 3-methyl flavones using iodine in dimethyl sulphoxide reagent in presence of a drop of concentrated sulphuric acid (Scheme 1).[28]

Experimental

Materials

All melting points were determined with an Electro thermal model 9100 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrophotometer in $CDCl_3$ with TMS as internal standard. The ^{1}H chemical shifts are reported downfield from TMS. IR spectra were recorded on Shimadzu FTIR spectrophotometer. All solvents were dried distilled prior to use.

Typical experimental procedure for 6-chloro-4'-methoxy-3-methyl flavanone (2a)

To a mineral support silica gel (1 g, 60—230 mesh) 0.2 mL concentrated sulphuric acid was added and then it is evaporated to dryness for firm adsorption of H_2SO_4 on silica gel. To this, *α*-methyl-2'-hydroxychalcone (**1a**) (0.1 mmol) was added with 5 mL dry dichloromethane. The reaction mixture was stirred at room temperature for 3 h (the reaction was monitored by TLC). Then it is filtered and the solvent was evaporated to obtain solid product, which is recrystallized by ethyl acetate. Light yellow solid. m.p. 101—102 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 7.85—7.81 (m, 1H, ArH), 7.41—7.34 (m, 2H, ArH), 7.24 (s, 1H, ArH), 6.96—6.89 (m, 3H, ArH), 5.00 (d, *J*=12 Hz, 1H, CH), 3.83 (s, 3H, OMe), 3.06—2.95 (m, 1H, CH), 1.01 (d, *J*=6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) *δ*: 193.54, 160.14, 159.70, 135.62, 129.64, 128.66, 126.88, 126.52, 121.08, 119.65, 115.71, 114.16, 114.42, 85.39, 55.31, 46.10, 10.19; IR (KBr) *v*: 3070 (Ar-H), 2982 (Aliph-C-H), 1681 (C=O), 1307 (C-O-C) cm^{-1}; MS (*m*/*z*): 302 (M + ion). Anal. calcd for $C_{17}H_{15}ClO_3$: C 67.44, H 4.99; found C 67.27, H 4.78.

Antibacterial activity

The antibacterial activities of compounds **2a**—**2d** and reference antibiotics were evaluated against the *Staphylococcus areus*, *Micrococcus luteus* and *Staphylococcus epidermis* by turbidometric method.[29-31] The inocula of microorganisms were prepared from 12 h grown culture. The tested samples were first dissolved in dimethyl sulphoxide (DMSO), then in Nutrient Broth to the highest dilution of 25 μg in each well of 96 wells micro plate.

The final concentration of DMSO in well was less than 1% (*ϕ*). Gentamycin and Penicillin diluted prior in water were used as reference. The bacterial inocula applied contained approximately 1.0×10^5 cells in a final volume of 200 μL of broth medium.

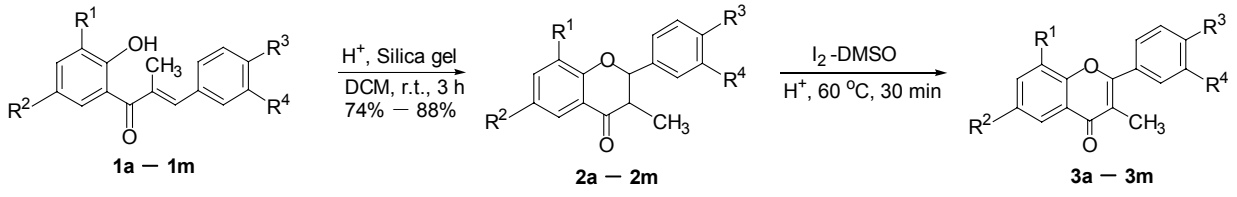
The micro plates were incubated for 24 h at 37 ℃ and optical density was measured at 600 nm. Values are used for calculation as means of three parallel experiments. IC_{50} values of the tested samples against pathogens were theoretically determined.

Results and Discussion

In present work we have successfully implemented cyclization technique for synthesis of substituted flavanone derivatives **2a—2m** from the substituted chalcones **1a—1m** (Scheme 1). The starting materials *α*-methyl-2'-hydroxychalcones were prepared from various substituted acetophenones and substituted benzaldehydes by using methanol and 40% sodium hydroxide solution. The 3-methyl flavanones were best prepared by cyclization of *α*-methyl-2'-hydroxychalcones in dichloromethane and sulphuric acid with silica gel support at room temperature. The acid catalyzed cyclization in presence of silica gel support faster and gave good yield of the flavanones. The method developed was simple and economical. The reaction was clean and no byproducts were seen. Since there is no heating during the entire course of reaction, it reduces side reactions and decomposition of products. The yields of the products obtained were comparable to other methods of the synthesis of flavanones. Compounds **2a—2m** gave positive test for flavanones. It did not give positive ferric chloride test indicating absence of hydroxyl group.

Recently we have developed a simple method for the synthesis of flavones by using iodine and dimethyl sulphoxide reagent. 2'-Allyloxychalcones were oxidatively cyclized to flavones by using catalytic amount of iodine in dimethyl sulphoxide.[28] Using this methodology we have converted flavanones **2a—2m** to flavones

Scheme 1 Synthesis of 3-methyl flavanones and its oxidation to 3-methyl flavones using I_2-DMSO reagent



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in good yields (Table 1). Flavanones **2a—2m** were heated with catalytic amount of iodine and dimethyl sulphoxide in the presence of a drop of concentrated sulphuric acid. The oxidation reaction was completed within 30 min to yield the flavones **3a—3m** (Table 2).

Table 1 3-Methyl flavanones obtained from α-methyl 2'-hydroxychalcones using H^+ with silica gel as a mineral support

Compd.	R^1	R^2	R^3	R^4	Yield of **2**[a,b]/%
2a	H	Cl	OCH_3	H	87
2b	H	CH_3	OCH_3	H	86
2c	H	Cl	OCH_3	OCH_3	88
2d	H	CH_3	OCH_3	OCH_3	82
2e	H	Cl	H	OCH_3	83
2f	H	Cl	H	Cl	81
2g	H	Cl	H	H	79
2h	H	Cl	$OCH_2CH=CH_2$	CH_3	74
2i	Cl	Cl	H	Cl	78
2j	Cl	Cl	OCH_3	OCH_3	81
2k	Cl	Cl	H	OCH_3	80
2l	Cl	Cl	H	H	78
2m	Cl	Cl	$OCH_2CH=CH_2$	OCH_3	75

[a] Isolated yields of the products. [b] Products are characterized by spectral analysis.

Table 2 Synthesis of flavones by oxidation of 3-methyl flavanones using I_2-DMSO reagent

Compd.	R^1	R^2	R^3	R^4	Yield of **3**[a,b]/%
3a	H	Cl	OCH_3	H	91
3b	H	CH_3	OCH_3	H	89
3c	H	Cl	OCH_3	OCH_3	87
3d	H	CH_3	OCH_3	OCH_3	92
3e	H	Cl	H	OCH_3	93
3f	H	Cl	H	Cl	92
3g	H	Cl	H	H	94
3h	H	Cl	$OCH_2CH=CH_2$	CH_3	91
3i	Cl	Cl	H	Cl	93
3j	Cl	Cl	OCH_3	OCH_3	92
3k	Cl	Cl	H	OCH_3	90
3l	Cl	Cl	H	H	85
3m	Cl	Cl	$OCH_2CH=CH_2$	OCH_3	87

[a] Isolated yields of the products. [b] Products are characterized by spectral analysis.

The ^{1}H NMR spectra of compounds **2a—2m** showed three prominent proton peaks near δ 1.01, 3.06 and 5.00. The spectra of compound **2a** showed doublet of 3-methyl proton at δ 0.98—1.01 (J=6.6 Hz). There was multiplet of C^3-H proton at δ 2.95—3.06 (J=7.1 Hz). The spectra also showed doublet of C^2-H proton at δ 4.96—5.00 (J=12.1 Hz). The compounds **2b—2m** showed nearly same values of proton peaks. The values of coupling constant confirm that C^2-H and C^3-H protons are *trans* in geometry. The downfield absorptions of C^2-H and C^3-H protons are due to their attachment to oxygen atom. A singlet of methoxy group was observed at δ 3.83. Aromatic protons showed multiplets in the downfield region of the spectra *i.e.* at δ 6.80—7.85. The IR spectra of compounds **2a—2m** showed absorptions nearly at 1307 cm^{-1} due to C—O—C stretching and 1681 cm^{-1} due to C=O stretching. The aliphatic C—H showed absorption at 2982 cm^{-1} and aromatic absorption at 3070 cm^{-1}.

The purity of compounds was checked by GC. The NMR spectra were consistent with the assigned structures.

Antimicrobial activity

Antimicrobial effects of flavone and flavanones and their derivatives have been extensively researched. It is well-known that many flavones and their derivatives exhibit antimicrobial activity. Therefore, our newly synthesized 3-methyl flavanones have been screened *in vitro* for antibacterial activity and structure activity relationship. The results obtained from antibacterial activity of flavanones are shown in Table 3. The flavanones **2a—2d** at the tested concentration exhibit antibacterial activity. Higher growth inhibition values were observed for flavanones against bacterial species *S. aureus*. The lowest IC_{50} values, which indicate the high antibacterial activity, were found against the *S. aureus* strain by **2c** and **2d**. Compound **2c** is more active against *Staphylococcus aureus*, *Micrococcus luteus* and *Staphylococcus epidermis*.

Structure-activity relationship suggests that modification in flavanones at R^4 position by OCH_3 was responsible for the higher antibacterial activity. The presence of Cl group at R^1 position indicates increase in activity of flavanones.

Table 3 Antimicrobial activity of 3-methyl flavanones as IC_{50} values against *Staphylococcus aureus*, *Micrococcus luteus* and *Staphylococcus epidermis*

Compd.	IC_{50}/(μmol•L^{-1})		
	Staphylococcus aureus	*Micrococcus luteus*	*Staphyloccus epidermis*
2a	109.11	171.59	154.52
2b	147.4	258.45	392.95
2c	44.35	79.50	120.90
2d	64.21	187.45	266.35
Gentamycin	29.56	23.87	—
Penicillin	—	—	84.16

[a] Isolated yields of the products. [b] Products are characterized by spectral analysis.

Conclusions

The 3-methyl flavanones were easily prepared by

cyclization of α-methyl-2'-hydroxychalcones in dichloromethane and sulphuric acid with silica gel support at room temperature. The method developed for synthesis of flavanones is economical because of simple procedure and absence of byproducts. And the newly synthesized 3-methyl flavanones containing Cl (R^1) and OCH_3 (R^4) showed higher antibacterial activity.

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