Reaction of active methylene compounds with 4-fluorobenzalanilines and antifungal potential of the products

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Abstract : Condensation of active methylene compounds (1-6) with 4-fluorobenzalaniline resulted in the formation of addition-elimination products 1a-6a respectively. The products were identified on the basis of elemental analysis, m.m.p. determination and spectral studies and were screened *in vitro* for their antifungal potential against four phytopathogenic fungi.

Keywords : Methylene compounds, antifungal agents.

In continuation of our work on substitution on benzalaniline and biological activity of the products, in this paper we have presented our study on the effect of fluoro substituent in the para position of C-phenyl ring of benzalanilines on the course of the condensation of active methylene compounds and to evaluate the products for their antifungal potential¹.

The reaction of cyanoacetic acid with 4-fluorobenzalaniline yielded a crude product which was recrystallized from ethanol. The product was identified as 4-fluorobenzalcyanoacetic acid (1a) with support of elemental analysis, IR and PMR studies. The infrared spectrum of the compound showed absorption bands at 2235 and 1710 cm⁻¹ indicating the presence of cyano group and carboxylic carbonyl respectively. The absorption at 1590 and 870 cm⁻¹ showed the presence of -CH=C< linkage. The five proton multiplet between δ 7.0-8.4 in the PMR spectrum of 1a in CDCl₃ accounted for four aromatic protons and one olefinic proton in 1a. Determination of m.m.p. (no depression) with an authentic sample of the compound prepared by alternative route further confirmed the structure of 1a. The result of this reaction is at variance as compared to the reaction of cyanoacetic acid with benzalaniline where addition product has been reported¹.

Condensation of malononitrile with 4-fluorobenzalaniline gave a crude solid which was recrystallized from methanol. The pure solid thus obtained was identified an addition-elimination product viz. 4-fluorobenzalmalononitrile (2a) on the basis of elemental

analysis, m.m.p. determination (no depression) with authentic sample and IR studies. The infrared spectrum of the compound showed absorption at 2230, 1585 and 840 cm⁻¹ indicating the presence of >C=N- and -CH=C< linkage respectively. The result of this reaction is similar to what has been reported in case of reaction of malononitrile with benzalaniline¹.

Treatment of 4-fluorobenzalaniline with ethyl cyanoacetate resulted in the formation of a crude solid which was recrystallized from benzene. The product was identified as ethyl 4-fluorobenzalcyanoacetate (3a), an addition-elimination product, with the help of elemental analysis and m.m.p. determination (no depression) with an authentic sample. This structure assignment for 3a was further confirmed on the basis of IR and PMR studies. The infrared spectrum contained absorption bands at 2230 and 1725 cm⁻¹ indicating the presence of cyano and ester group respectively. The absorption at 1590 and 850 cm⁻¹ showed the presence of -CH=C< linkage. The PMR spectrum of 3a in CDCl₃ has indicated ethyl protons of the ester group as a two proton quartet at δ 4.4 and a three proton triplet at δ 1.5 and four aromatic protons alongwith one olefinic proton as a multiplet between δ 6.9-8.3. The result of this reaction is at variance in comparison to such a study with benzalaniline where two products viz. α -cyano- β -anilinodihydrocinnamic acid, an addition product with hydrolysed ester group (10%) and ethyl benzalcyanoacetate, an addition-elimination product (77%) have been reported¹.

When ethyl acetoacetate was allowed to react with 4-

fluorobenzalaniline, a solid separated out. The solid after recrystallization from benzene was identified as ethyl 4-fluorobenzalacetoacetate (**4a**) with the support of elemental analysis, m.m.p. determination and spectral studies. The infrared spectrum of the compound showed bands at 1720 and 1590 and 855 cm^{-1} indicating the presence of ester group and $-\text{CH}=\text{C}<$ linkage respectively. The PMR spectrum of **4a** in CDCl_3 has shown a five proton multiplet between δ 6.8–8.3 for four aromatic protons and one olefinic proton, a three proton singlet at δ 2.4 for CH_3 protons of acetyl group and a two proton quartet at δ 4.5 along with a three proton triplet at δ 1.4 for C_2H_5 proton of ester group.

The crude product obtained by the condensation of acetylacetone with 4-fluorobenzalaniline was recrystallized from benzene. The compound was identified as 4-fluorobenzalacetylacetone (**5a**) on the basis of elemental analysis, IR and PMR studies. The infrared spectrum of the compound indicated absorption bands at 1740 and 1590 and 860 cm^{-1} which were assigned to carbonyl group and $-\text{CH}=\text{C}<$ linkage respectively. In the PMR spectrum of **5a** in CDCl_3 , the six proton singlet at δ 2.5 was assigned to methyl protons of acetyl groups and the five proton multiplet between δ 6.9–8.3 was due to four aromatic protons and one olefinic proton. The structure was further confirmed with the support of m.m.p. determination (no depression) with the authentic sample of the compound prepared by an alternative route. The result of this study is different from that of benzalaniline where an addition product has been reported.

The condensation of nitromethane with 4-fluorobenzalaniline yielded a crude solid which was recrystallized from benzene. The product was characterized as 4-fluorobenzalnitromethane (**6a**), an addition-elimination product with the support of elemental analysis, m.m.p.

determination (no depression) with authentic sample² and IR studies. The infrared spectrum of the compound contained absorption bands at 990, 1540 and 1360 cm^{-1} indicating the presence of $-\text{CH}=\text{C}<$ linkage and nitro group respectively.

The presence of fluoro substituent in the para position of C-phenyl ring of benzalaniline it seems, facilitates the formation of addition-elimination products rather than addition products. The carbanion formed from the active methylene compounds adds to the carbon-nitrogen double bond of 4-fluorobenzalaniline to give unstable addition product which loses the aniline molecule to yield stable addition-elimination product (Scheme 1). The 4-fluorobenzal derivatives alongwith their characteristics are recorded in Table 1.

The 4-fluorobenzal derivatives **1a–6a** were screened *in vitro* for their antifungal potential against *Alternaria alternata*, *Fusarium oxysporum*, *Colletotrichum capsici* and *Myrothecium roridum* by spore germination inhibition method at various concentrations. The results have been expressed in terms of ED_{50} values in Table 2.

Scheme 1

Table 1. Characteristics of 4-fluorobenzal derivatives

Compd.	A	B	M.p. ^a (°C)	Yield (%)	Molecular formula ^b
1a	CN	COOH	104	38	$\text{C}_{10}\text{H}_6\text{O}_2\text{NF}$
2a	CN	CN	130	40	$\text{C}_{10}\text{H}_5\text{N}_2\text{F}$
3a	CN	COOC_2H_5	88	35	$\text{C}_{12}\text{H}_{10}\text{O}_2\text{NF}$
4a	COCH_3	COOC_2H_5	132	30	$\text{C}_{13}\text{H}_{13}\text{O}_3\text{F}$
5a	COCH_3	COCH_3	70	40	$\text{C}_{12}\text{H}_{11}\text{O}_2\text{F}$
6a	H	NO_2	61	42	$\text{C}_8\text{H}_6\text{NO}_2\text{F}$

^aThe melting points are uncorrected.

^bThe compounds gave satisfactory elemental analysis.

Table 2. Antifungal potential of 4-fluorobenzal derivatives

Compd.	ED ₅₀ values (ppm) against			
	<i>A. alternata</i>	<i>F. oxysporum</i>	<i>C. capsici</i>	<i>M. roridum</i>
1a	750	690	310	*
2a	*	*	*	*
3a	*	*	360	160
4a	140	830	*	85
5a	*	*	*	*
6a	*	910	*	*
Dithane M-45	30	–	12	32
Bavistin	–	7	–	–

* More than 1000 ppm.

Two of the compounds (2a and 5a) have ED₅₀ values more than 1000 ppm against all the test fungi. Three compounds (1a, 4a and 6a) have ED₅₀ values less than 1000 ppm against *F. oxysporum*. Two compounds 1a and 3a have better antifungal potential against *C. capsici* with ED₅₀ value of 310 and 360 ppm. Ethyl 4-fluorobenzalacetoacetate (4a) has been found to possess promising antifungal potential against *M. roridum* and *A. alternata* with ED₅₀ value of 85 ppm and 140 ppm respectively. The compound 3a namely ethyl 4-fluorobenzalcyanoacetate also possesses promising activity against *M. roridum* with ED₅₀ value of 160 ppm.

Experimental

Reaction of active methylene compounds with 4-fluorobenzalaniline :

4-Fluorobenzalaniline (0.01 mol) was dissolved in benzene (20 ml) in a conical flask (100 ml). Active methylene compound (0.01 mol) was added to the above solution. Then a few drops of pyridine were added. The mixture was warmed with stirring, cooled, stoppered and allowed to stand at room temperature overnight when a

crude solid separated out. The crude product was filtered and recrystallized from suitable solvent to get respective 4-fluorobenzal derivative (1a-6a).

In vitro screening for antifungal potential :

The stock solution of each compound was prepared by dissolving each chemical (20 mg) in absolute alcohol (0.5 ml) and the volume was made to 10 ml by adding sterilized distilled water. The stock solution of 2000 ppm of each compound, thus, prepared on active ingredient basis was kept in refrigerator till further use. The required dilutions of 1000, 500, 250, 100 and 50 ppm were subsequently made from the stock solution by adding sterilized distilled water as and when required.

Small droplets (0.02 ml) of test solution and spore suspension in equal amount were seeded in the cavity slides. These slides were kept in petriplates lined with moist filter paper and then incubated for 24 h at 25 ± 1 °C. The slides were checked for percent germination occurred and percent spore germination inhibition was calculated and ED₅₀ values were determined.

References

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