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Chemoselective reaction of cyanoacetic acid with benzal-4-acetylanilines and fungitoxicity of products

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Abstract. Cyanoacetic acid reacted chemoselectively with carbon–nitrogen double bond of benzal-4-acetylaniliines, leaving the carbon–oxygen double bond, considered to be more reactive, intact, leading to the formation of mono addition–elimination products rather than *bis* attack at both the reactive centres, even when the reaction was carried out with two moles of cyanoacetic acid. The product viz. benzal-cyanoacetic acid and its derivatives were screened for their fungitoxicity against five pathogenic fungi.

Keywords. 4-Aminoacetophenone; cyanoacetic acid; benzal-4-acetylanilines; benzalcyanoacetic acid; fungitoxicity.

1. Introduction

Chemistry of multiple bonds has achieved a dramatic development in the past decades[1–4] because these compounds have been used as substrates in the synthesis of industrial and biological active compounds via ring closure,[5–6] cycloaddition[7–8] and condensation reactions.[9–11] Moreover, the compounds containing carbon–nitrogen, carbon–oxygen and/or carbon–carbon double bond are also known to possess biological activity.[4,11–14] Reaction of cyanoacetic acid with carbonyl compounds yields condensation products[15] whereas such a reaction with imines, the compounds containing carbon-nitrogen double bond, results in the formation of various products viz. adducts,[16] addition–elimination products,[17] coumarinimide derivatives[5], etc. depending on the starting imine and the reaction conditions. The carbon–nitrogen double bond is intermediate[18] in its reactivity between carbonyl compounds and alkenes. The present work was aimed to study the reaction of cyanoacetic acid with benzal-4-acetylaniline and its C-phenyl derivatives, the compounds containing both carbon–nitrogen double bond as well carbon–oxygen double bond and unusual results of this work along with fungitoxicity of the products are being presented in this paper.

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2. Experimental

The melting points were determined on electrical melting point apparatus and are uncorrected. Purity of the compounds was checked by TLC. The compounds gave satisfactory elemental analysis. The IR spectra were recorded on a Perkin Elmer FT–IR spectrometer using KBr disc. The PMR spectra were recorded on a Brucker Spectrospin 300 MHz spectrometer in $CDCl_3$ with TMS as internal standard. Mass spectra were recorded on Perkin Elmer Clarus 500 Mass Spectrometer.

2.1 *General procedure for the reaction of cyanoacetic acid with benzal-4-acetylanilines*

Benzal-4-acetylaniline/its derivative (1a–10a) (0·01 mol) was taken in dry benzene (20 ml) in a conical flask (100 ml). Then cyanoacetic acid (0·01 mol) and a few drops of pyridine were added to the above solution. The reaction mixture was heated and shaken briskly for 20 min. The flask was then cooled, stoppered and allowed to stand at room temperature for 24 h. when a crude solid separated out which was filtered and recrystallized from benzene to get pure benzalcyanoacetic acid/its derivative (1b–10b). Evaporation of the solvent from the filtrate yielded gelly like mass, TLC of which indicated the presence of 4-aminoacetophenone and unreacted starting materials.

Condensation of cyanoacetic acid with benzal-4-acetylanilines (1a–10a) in 2 : 1 molar ratio was also carried out by following the above procedure.

2.2 *In vitro screening for fungitoxicity*

Stock solution of the test compounds and standard fungicides, viz. Indofil M-45 (75% manganese ethylene bisdithiocarbamate + 2% Zinc ion), Bavistin 50 WP (methyl-2-benzimidazole carbamate) and Vitavax 75 WP (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) were prepared by dissolving each chemical (20 mg) in absolute alcohol (0·5 ml) and making up the final volume to 10 ml with sterilized distilled water. Stock solutions of 2000 ppm thus prepared on active ingredient basis were kept in refrigerator till further use to prepare solution of required concentration.

Actively growing ten-day old cultures of the test fungi except *U. tritici* were taken from PDA slants and the spore suspension was made by addition of sterilized distilled water. The suspension was filtered through three layers of sterilized cheese cloth under aseptic conditions in order to remove agar bits and mycelium. Haemocytometer was used to get spore suspension (1×10^6 spore/ml). Screening of the test compounds against *U. tritici* involved floating of fungal spores on the surface of test solution in cavity slides.

Small droplets (0·02 ml) of the 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 incubated for 24 h at 25 ± 1°C. The slides were checked for germination and per cent spore germination inhibition was determined from which ED_{50} values were calculated using Polo Software Program.

3. Results and discussion

Condensation of cyanoacetic acid with benzal-4-acetylanilines (1a–10a), the compounds synthesized by reaction of 4-aminoacetophenone with benzaldehyde and substituted benzaldehydes (1–10), in equimolar ratio in the presence of pyridine yielded crude solids (1b–10b) which were purified by recrystallization from benzene.

3.1 *Spectral analysis*

The IR spectra of the products contained absorption bands at 2225 and 1730 cm^{-1} depicting the presence of cyano and carboxylic carbonyl group respectively. The absorption at 1590 and 850 cm^{-1} was due to –CH = C< linkage. In addition to the above bands, the absorption bands of functional group of the respective product were also observed. For instance, the absorption band between 3400 and 3300 cm^{-1} in the compounds 3b, 7b and 8b was indicative of presence of phenolic group in these products. In the IR spectra of products 9b and 10b, the bands between 1540 and 1500 and 1360–1300 cm^{-1} were due to nitro group.

In the PMR spectra of the products, the protons resonated in the expected field. Multiplet signals of integration corresponding to six protons in compound 1b; five protons in the products 2b, 3b, 4b, 9b and 10b; four protons in the compounds 5b, 7b and 8b and three protons in the product 6b observed between δ 7·0 and 7·9 accounted for aromatic protons alongwith one olefinic proton. A singlet at about δ 4·0 corresponding to three protons in compounds 4b and 7b, six protons in compound 5b and nine proton in compound 6b indicated the presence of methoxy protons in these products. A one proton singlet at δ 9·5 in compounds 3b, 7b and 8b showed the presence of phenolic proton. A two proton quartet at δ 4·4 and three proton triplet at δ 1·5 in the PMR spectrum of compound 8b was due to the protons of ethoxy group. Mass spectra of the products revealed that the molecular ion peak also constituted the base peak.

On the basis of analytical and spectral data, the products have been characterized as benzal-cyanoacetic acid and its derivatives. The benzal-cyanoacetic acid derivatives alongwith their physical characteristics and molecular ion peaks are recorded in table 1. Reaction of cyanoacetic acid with 1a–10a in 2 : 1 molar ratio also yielded the same products 1b–10b respectively. The formation of benzalcyanoacetic acid and its derivatives can be explained by the attack of the carbanion formed from cyanoacetic acid on carbon–nitrogen double bond of benzal-4-acetylanilines to give unstable addition products which lose 4-aminoacetophenone to yield the stable addition–elimination products (scheme 1).

Cyanoacetic acid thus, reacted chemoselectively with azomethine linkage of benzal-4-acetylanilines (1a–10a) leaving the ketonic moiety, considered to be more reactive, intact under reaction conditions, leading to the formation of mono addition–elimination products rather than *bis* attack at both the reactive centers, viz. carbon–nitrogen as well as carbon–

Table 1. Physical characteristics and molecular ion peaks of benzalcyanoacetic acid.

Compound	R	Melting point (°C)	Yield (%)	Elemental analysis calculated % (found) C	H	N	M^+ (*m/z*)	Molecular formula
1b	H	46	88	69·36 (69·40)	4·05 (4·00)	$C_{10}H_7NO_2$	173	$C_{10}H_7NO_2$
2b	4-Cl	105	68	57·83 (57·90)	2·89 (2·85)	$C_{10}H_6NO_2Cl$	207	$C_{10}H_6NO_2Cl$
3b	4-OH	118	75	63·49 (63·57)	3·70 (3·65)	$C_{10}H_7NO_3$	189	$C_{10}H_7NO_3$
4b	4-OCH_3	126	67	65·02 (64·95)	4·43 (4·40)	$C_{11}H_9NO_3$	203	$C_{11}H_9NO_3$
5b	3-OCH_3 4-OCH_3	102	79	61·80 (61·90)	4·72 (4·70)	$C_{12}H_{11}NO_4$	233	$C_{12}H_{11}NO_4$
6b	3-OCH_3 4-OCH_3 5-OCH_3	108	85	59·32 (59·30)	4·94 (5·00)	$C_{13}H_{13}NO_5$	263	$C_{13}H_{13}NO_5$
7b	3-OCH_3 4-OH	118	80	60·27 (60·35)	4·11 (4·10)	$C_{11}H_9NO_4$	219	$C_{11}H_9NO_4$
8b	3-OC_2H_5 4-OH	68	82	61·80 (61·85)	4·72 (4·65)	$C_{12}H_{11}NO_4$	233	$C_{12}H_{11}NO_4$
9b	2-NO_2	58	63	55·05 (54·95)	2·75 (2·70)	$C_{10}H_6N_2O_4$	218	$C_{10}H_6N_2O_4$
10b	3-NO_2	98	60	55·05 (55·10)	2·75 (2·70)	$C_{10}H_6N_2O_4$	218	$C_{10}H_6N_2O_4$

*The m.p. were determined on electric melting point apparatus and are uncorrected

Table 2. Antifungal potential of benzalcyanoacetic acids.

Compound	ED_{50} values (ppm) against *A. alternata*	*C. capsici*	*F. oxysporum*	*M. roridum*	*U. tritici*
1b	370	120	250	110	170
2b	*	100	240	70	260
3b	*	180	380	*	*
4b	170	72	170	150	*
5b	*	106	360	640	960
6b	720	220	760	80	900
7b	670	740	*	350	940
8b	370	650	740	*	*
9b	*	*	*	*	*
10b	*	960	*	252	*

*More than 1000 ppm

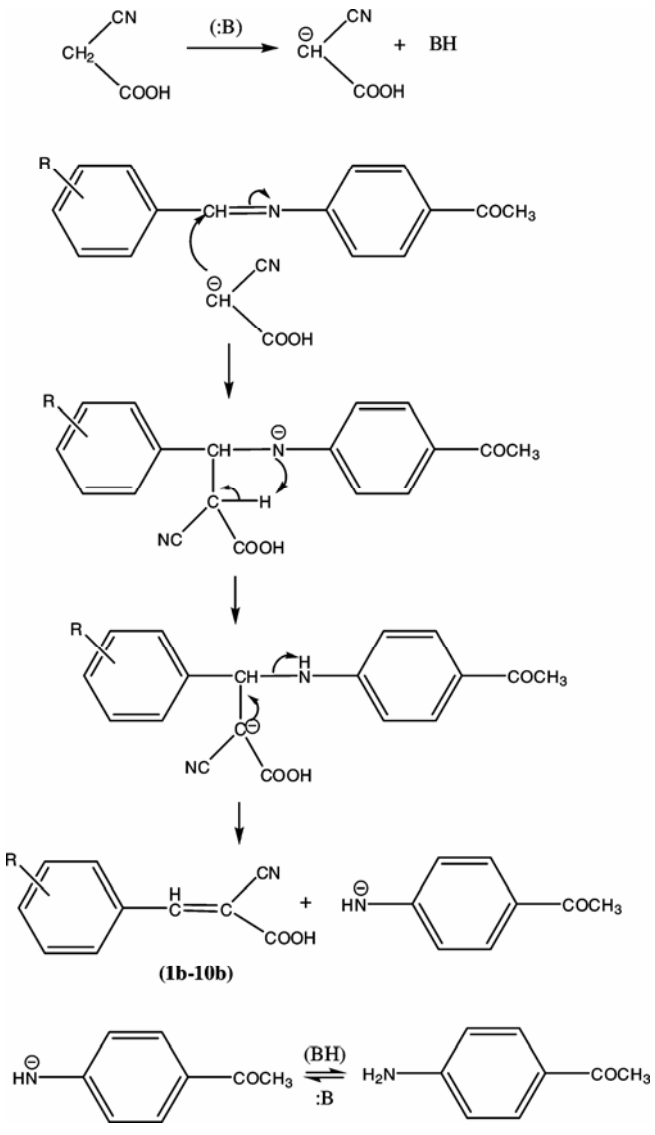
oxygen double bond even when the reaction was carried out with two moles of cyanoacetic acid.

3.2 *Fungitoxicity of products*

Benzalcyanoacetic acid and its derivatives, (1b–10b) were evaluated *in vitro* for their fungitoxicity against *Alternaria alternata, Colletotrichum capsici*, *Fusarium oxysporum*, *Myrothecium roridum* and *Ustilago tritici* by spore germination inhibition method[19] at various concentrations. The results have been expressed in terms of ED_{50} values i.e. the effective dose at which 50 per cent spore germination inhibition was caused (table 2). Five of the test compounds possessed ED_{50} values less than 1000 ppm against *A. alternata* and the most effective among these was found to be 4-methoxybenzalcyanoacetic acid (4b) with ED_{50} value of 170 ppm. All the test compounds except 9b had ED_{50} values less than

1000 ppm against *C. capsici* and the most potent among there was 4-methoxybenzalcyanoacetic acid (4b) with ED_{50} value of 72 ppm. Among the seven test compounds which showed ED_{50} values less than 1000 ppm against *F. oxysporum,* again 4-methoxybenzalcyanoacetic acid (4b) exhibited maximum potential with ED_{50} value of 170 ppm.

The test compounds except 3b, 8b, 9b, possessed ED_{50} values less than 1000 ppm against *M. roridum* and the compound namely 4-chlorobenzalcyanoacetic acid (2b) showed the best results with ED_{50} value of 70 ppm. The test compounds were less effective against *U. tritici* and benzalcyanoacetic acid (1b) was the only compound which showed promising activity with ED_{50} value of 170 ppm.



Scheme 1.

4. Conclusions

Cyanoacetic acid reacted with carbon–nitrogen double bond of benzal-4-acetylanilines only leaving carbon–oxygen double bond, considered to be more reactive, intact under the reaction conditions. Thus condensation of cyanoacetic acid is a chemoselective reaction with benzal-4-acetylanilines.

4-Methoxybenzalcyanoacetic acid (4b) was found to be most potent among the test compounds against *A. alternata*, *C. capsici* and *F. oxysporum* with ED_{50} value of 170, 72 and 170 ppm, respectively. The most effective compound against *M. roridum* and *U. tritici* was 4-chlorobenzalcyanoacetic acid (2b) and benzalcyanoacetic acid (1b) respectively with ED_{50} value of 70 and 170 ppm.

Introduction of nitro-substituent in the phenyl ring of benzalcyanoacetic acid resulted in sharp decline in the fungitoxicity of the parent compound. Thus, the nitro-substituted derivatives (9b, 10b) possessed ED_{50} values of more than 1000 ppm against the test fungi in most of the cases.

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