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Chemical and Spectrophotometric Investigations of 3-Mercapto-5,6-diphenyl-1,2,4-triazine as a New Carboxyl Activating Group

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The synthesis of 3-mercapto-5,6-diphenyl-1,2,4-triazine and its benzoyl derivatives and the subsequent conversion of respective amides and esters using chemical as well as spectrophotometric tools are described. This paved the way for the introduction of a new and versatile carboxyl activating group which was more effective than conventional carboxyl activating groups that usually apply in the solid phase peptide synthesis.

Key Words: Carboxyl activating group, Triazine thiols, Acyl carrier protein.

INTRODUCTION

Synthesis of a peptide with a well defined sequence of amino acid residues is fairly a complex process. The major problem associated with peptide synthesis lies in the formation of these amides or peptide bonds that couple amino acids. The synthesis of naturally occurring peptides and their structural analogues for biological and pharmacological investigations necessitates the availability of efficient and modified peptide synthesis strategies.

A number of heterocyclic systems, having a thiol function have been proved to be effective in carboxyl group activation[1-4]. Moreover, new methods have been developed for the preparation of thiol esters. Active role of thiol esters in biochemical processes and their reactivity with various nucleophiles has led investigators to choose them as attractive synthetic alternatives in organic synthesis. Carboxyl group activated heterocyclic compounds, possess enhanced reactivity, which promotes synthesis under mild conditions. This has been found to provide effective synthetic methods for peptides[5,6], macrolides[7,8] and carbohydrates[9,10]. In recent years there has been tremendous advance in the chemistry of heterocyclic compounds[11]. Among heterocyclic compounds, triazines have drawn much attention as plant growth regulators[12-17] disinfectants[18] and bleaching agents[19]. Therefore, it is thought worthwhile to investigate the role of trazine as carboxyl activating group in organic synthesis. Thus, the synthesis of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**1**) which can easily be tautomerized to thione function (**1'**) to serve as a novel carboxyl activating group under mild conditions (**Chart-1**) and its reaction with different amines, alcohols and amino alcohols have been carried out so as to illustrate the usefulness of the carboxyl activating capability of 3-mercapto-5,6-diphenyl-1,2,4-triazine (3-MDT).

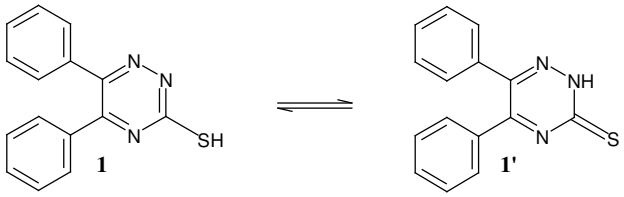


Chart-1

EXPERIMENTAL

TLC was carried out by using precoated silica gel plates. In column chromatography (100 cm × 2 cm) neutral alumina and silica gel were used as adsorbants and the solvent systems used were petroleum ether-ethyl acetate (4:1), chloroform, methanol and water. UV-visible spectra were recorded on a Shimadzu UV-1601 spectrophotometer. IR spectra were recorded on Shimadzu IR-470 spectrophotometer using KBr pellets. ^{1}H NMR and ^{13}C NMR and mass spectral measurements were recorded elsewhere.

Synthesis

3-Mercapto-5,6-diphenyl-1,2,4-triazine (3-MDT) (1): A solution of benzil (4.2 g, 0.01 mol) in ethanol (60 mL) was refluxed and to the refluxing solution an equimolar solution of thiosemicarbazide (1.82 g, 0.02 mol) in water (12 mL) was added. To the reaction mixture, ammonium acetate crystals were added till the solution became turbid and the mixture was refluxed for 10 h, cooled in ice and the crystals separated were filtered. Recrystallization from alcohol afford yellow crystals of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**1**), Yield 2.52 g (95 %), m.p. 205 ºC.

N-Benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (N-BzDTT) (2'): The benzoylation of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**1**) was carried out by Schotten Baumann method. To a solution of **2** (2.65 g) in 10 % NaOH, benzoyl chloride (0.25 mL) was added in drops with thorough shaking. The sparingly soluble benzoyl derivative was separated out as pale yellow solid. Filtered, washed with cold water and then recrystallized from ethanol to get pale yellow crystals of N-BzDTT (**2'**) Yield 2.77 g (75 %), m.p. 125 ºC.

DCC coupling method was also adopted to prepare N-BzDTT (**2'**). In a typical procedure a solution of benzoic acid (1.22 g, 10 mmol) and 3-mercapto-5,6-diphenyl-1,2,4-triazine (**2**, 2.65 g) in a 1:4 mixture of THF and CH_2Cl_2 (10 mL), was treated with DCC (10 mmol) in methylene chloride (5 mL) with constant stirring at < 5 ºC for *ca.* 1 h. The precipitated DCU was filtered off. The filtrate was concentrated and separated by column chromatography using silica gel column. Recrystallization from alcohol afforded yellow crystals of N-BzDTT. Yield 3.32 g (90 %), mp 125 ºC. Mixed melting point with the sample prepared by the Schotten-Baumann method did not show any depression.

Aminolysis of N-BzDTT formation of amides: To a solution of N-BzDTT (**2'**, 0.74 g, 2 mmol) in chloroform (10 mL), freshly distilled aniline (0.19 mL, 2 mmol) was added with constant stirring at room temperature. The orange red colour appeared

immediately deepened after 5 min. The reaction was followed by TLC and spectrophotometrically. The mixture was concentrated and then separated by column chromatography (neutral alumina). The first fraction separated was evaporated to dryness. White crystalline solid obtained was recrystallized from alcohol to afford benzanilide, Yield 3.35 g, (90 %), m.p. 162 ºC (lit.[20] m.p. 163 ºC). The other fraction separated was isolated and identified as 3-MDT almost in quantitative yield.

Similar aminolysis reactions were repeated with different amines so that respective amides were formed. In all the cases, 3-MDT (**1**) was regenerated in quantitative yield. The characterization details of the products formed are given in Table-1.

TABLE-1
ACYLATION OF AMINES/ALCOHOLS USING N-BzDTT

Amines/alcohols/ amino alcohols used	Amides/esters formed	m.p./b.p. (lit. m.p./ b.p.) (ºC)	Reaction time (min)	Yield (%)
Aniline (**3a**)	Benzanilide (**4a**)	162(163)[21]	7	90
2-Methyl aniline (**3b**)	N-Benzoyl-2-methyl aniline (**4b**)	141(143)[21]	5	85
4-Methyl aniline (**3d**)	N-Benzoyl-4-methyl aniline (**4d**)	154(156)[20]	5	90
4-Chloraniline (**3e**)	N-Benzoyl-4-chloraniline (**4e**)	190(193)[20]	10	65
4-Methoxy aniline (**3f**)	N-Benzoyl-4-methoxy aniline (**4f**)	150(152)[20]	6	75
Benzylamine (**3g**)	N-Benzoyl benzamide (**4g**)	106(106)[20]	8	70
Ethylamine (**3j**)	N-Ethyl benzamide (**4j**)	248	5	75
Methyl alcohol (**5a**)	Methyl benzoate (**6a**)	196(199)[20]	20	52*
Ethyl alcohol (**5b**)	Ethyl benzoate (**6b**)	211(212)[20]	20	47*
Benzyl alcohol (**5f**)	Benzyl benzoate (**6f**)	302(306)[21]	20	40*
Ethanol amine (**3m**)	N-(2-hydroxyethyl)benzamide (**4m**)	161(163)[20]	10	75
Diethanol amine (**3n**)	N,N-*bis*(2-hydroxyethyl)benzamide (**4n**)	150(153)[20]	10	65
p-Amino phenol (**3p**)	N-(4-hydroxyphenyl)benzamide (**4p**)	228(230)[20]	12	70

*Yield Calculated based on the amount of DTT regenerated

Reaction of N-BzDTT with alcohols: Formation of esters: N-BzDTT (**2'**, 1.48 g, 4 mmol) was dissolved in chloroform (20 mL) and treated with ethanol (25 mL). The mixture was heated to about 70 ºC, for 5 min and reaction mixture was concentrated and separated using a neutral alumina column to afford ethyl benzoate, Yield 47 % b.p. 211 ºC. The slow regeneration of **2** was also monitored spectrophotometrically. Esterification reaction was generalized by extending the reaction using different alcohols to afford the respective esters. The nature and course of reaction were similar to that of ethyl alcohol. Characterization data of the products formed are presented in Table-1.

Selective aminolysis of N-BzDTT with amino alcohols: N-BzDTT (**2'**, 0.74 g; 2 mmol) was dissolved in chloroform (10 mL). Ethanolamine (0.12 g, 2 mmol) was then added to it. Stirred thoroughly for *ca.* 5 min. Intense red orange colour was developed indicating the sudden regeneration of DTT (**2**). The reaction was monitored by TLC and spectrophotometrically. The products formed were separated

using neutral alumina column. The first fraction separated was concentrated and recrystallized from alcohol to afford N-(2-hydroxy ethyl)benzamide, yield (75 %), m.p. 161 ºC (lit[20]. m.p. 163 ºC). Similar reaction was carried out using diethanolamine to get the respective amide, N,N-*bis*(2-hydroxyethyl)benzamide.

UV-Visible absorption spectra of N-BzDTT during aminolysis and esterification: To carry out the spectrophotometric investigation on aminolysis of N-BzDTT (**2'**), a very dilute solution (0.1 mmol) was prepared in chloroform and taken in a cuvet to get the initial absorbance value. Then a 0.01 mmol solution of ethylamine in chloroform was transferred to the cuvet. Stirred well with the aid of a capillary tube and the absorbance value noted at definite intervals (Fig. 1a-d).

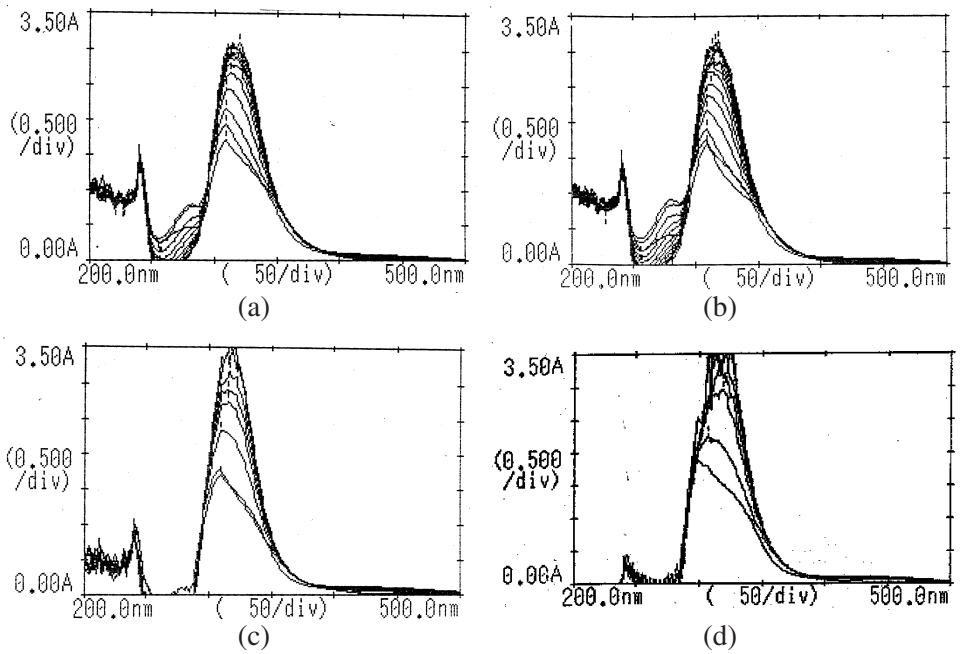


Fig. 1 (a) UV-Visible spectrum-Reaction of aniline with N-BzDTT (**2'**)
(b) UV-Visible spectrum-Reaction of *o*-toluidine with N-BzDTT (**2'**)
(c) UV-Visible spectrum-Reaction of *n*-propylamine with N-BzDTT (**2'**)
(d) UV-Visible spectrum-Reaction of *n*-butylamine with N-BzDTT (**2'**)

Electronic effects like inductive, mesomeric effects and the nucleophilicity of the amines or alcoholic group play an important role in the rate of different reactions. Therefore, in addition to the investigation on the extent of aminolysis and esterification of individual reactions, a comparative study on different amines, amino-alcohols, alcohols and phenols in order to make a qualitative correlation with the electronic arrangement of nucleophiles was also carried out.

Aliphatic and aromatic amines and hydroxyl compounds were used to study the rate of benzoylation and esterification using N-BzDTT (**2'**). The relative absorbance

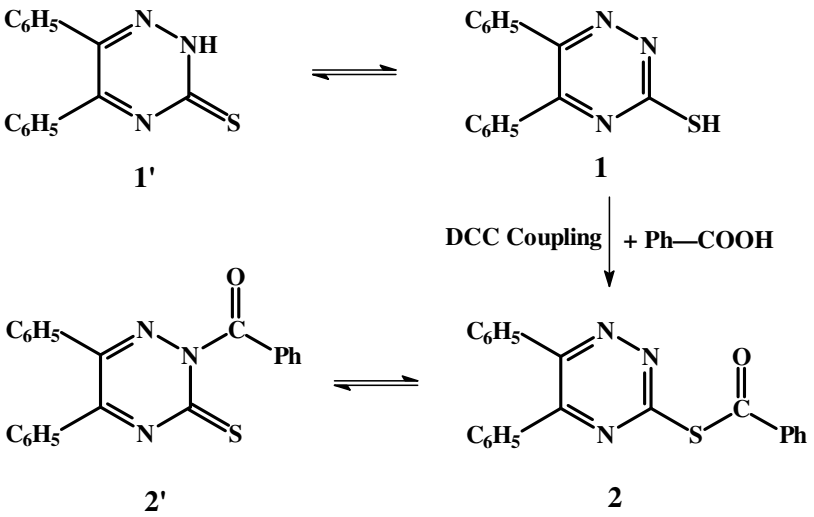
in aminolysis using various amines and amino alcohols has been plotted against time so that the extent of the reaction could be compared. From the figure, it is observed that aliphatic amines showed better reactivity than aromatic amines towards benzoylation. The electron releasing effect of the alkyl groups in aliphatic amines and consequent increase in nucleophilicity on nitrogen explains better aminolysis than that of aromatic amines. Amongst aliphatic amines, *n*-butyl amine showed the highest rate than that of *n*-propyl amine and ethyl amine, which is in agreement with the influence of electron releasing inductive effect of alkyl groups. Aniline showed the least reactivity towards benzoylation. Here electron withdrawing effect of the phenyl group would have influenced the reactivity at the nitrogen centre. In case of *m*-toluidine, a better reactivity than aniline may be due to the positive inductive effect of methyl group at the *meta* position which in turn makes the reaction faster than aniline. *o*-Ethylaniline showed more reactivity than *m*-toluidine, which could be due to the presence of electron donating ethyl group at the *o*-position of the benzene ring. In aliphatic polyfunctional compounds like ethylenediamine, ethanolamine and diethanolamine an increase in the reaction trend was observed. Increase in availability of electrons at the nitrogen centre offers the suitable explanation. An exceptional behaviour was observed in case of *p*-chloroaniline where a high reactivity was noticed. The esterification of N-BzDTT (**2'**) using alcohols and phenols are sluggish in nature, probably due to the less nucleophilicity of the hydroxy group than amino group.

RESULTS AND DISCUSSION

Benzoylation of 2-mercapto-5,6-diphenyl-1,2,4-triazine (1): 3-MDT was prepared using the established procedure. The benzoylation of (**1**) was carried out by Schotten Baumann reaction. In this method 1 m molar solution of (**1**) in 5 % NaOH (20 mL) was shaken vigorously with 0.5 mL benzoyl chloride, till the odour of benzoyl chloride disappeared. The solid benzoyl derivative N-BzDTT formed was recrystallized from ethanol to get pale yellow solid with m.p. 125 ºC in 85 % yield, which was characterized as N-BzDTT (**2'**) by IR, NMR and mass spectral methods.

Benzoylation of (**1**) was also done by DCC coupling method. Here an equimolar solution of benzoic acid and 3-mercapto-5,6-diphenyl-1,2,4-triazine (**1**) in THF and CH_2Cl_2 mixture (1:4), an equivalent amount of DCC in CH_2Cl_2 was added with constant stirring for 1 h in an ice bath. The product obtained was separated in silica gel column and was recrystallized from ethanol to afford pale yellow crystals with m.p. 125 ºC in 90 % yield. Mixed melting point with the compound obtained by Schotten-Baumann reaction did not show any depression and it was homogeneous to TLC (**Scheme-I**).

The formation of the N-benzoyl derivative **2'** from S-benzoyl-5,6-diphenyl-1,2,4-triazine (**2**) can be explained as an S $\rightarrow$ N thermal rearrangment of the kinetic product **2** to the thermodynamically more stable N-benzoyl-5,6-diphenyl-1,2,4-

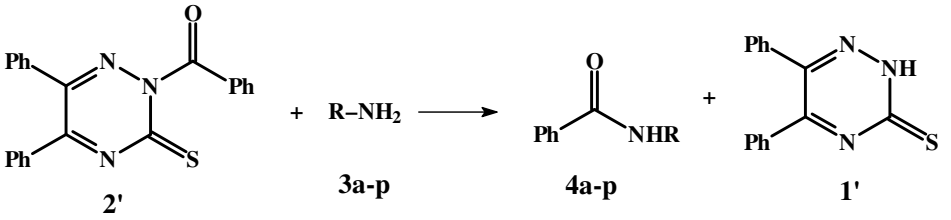


Scheme-I

triazine-3-thione (**2'**). Similar explanations were offered to the rearranged products observed in the case of acyl derivatives of 2-thionothiazolidenes. Hence both N-acyl (**2'**) and S-acyl (**2**) derivatives are reasonably possible.

Acylation of amines using N-BzDTT (Formation of amides): The suitability of 3-MDT (**1**) as carboxyl activating group was established by studying acylation reaction with simple amines. Thus, a chloroform solution of N-BzDTT (**2'**) was treated with an equimolar amount of freshly distilled amines or amino alcohols. Benzoylation reactions were very effective and rapid. The simultaneous regeneration of pale orange 3-MDT (**1**) was observed by TLC and spectrophotometric scannings. Initially, aminolysis reaction was carried out by treating a chloroform solution of the N-BzDTT (**2'**) with an equimolar amount of freshly distilled aniline (**3a**). The benzoylation was almost completed in 5 min. Regeneration of 3-mercapto-5,6-diphenyl-1,2,4-triazine (**1**) was observed by the colour change of the reaction mixture to pale red orange. The reaction mixture was concentrated by evaporation and separated by column chromatography (neutral alumina). The first fraction obtained was identified as benzanilide (**4a**) in 90 % with m.p. 162 ºC (lit. m.p. 163 ºC). Presence of benzanilide was further evidenced by TLC and mixed melting point with an authentic sample of benzanilide did not show any depression. The regenerated 3-MDT was also in quantitative yield. To establish the general nature of aminolysis the reaction was carried out with different amines (**Scheme-II**). All the reactions were carried out at room temperature. In all the cases, 3-MDT (**1**) was also regenerated in almost quantitative yield.

Acylation of alcohols using N-BzDTT(2') (Formation of esters): Formation of ester from carboxylic acid and alcohol is basically a reversible reaction and hence isolation of the desired product requires complicated purification procedures.

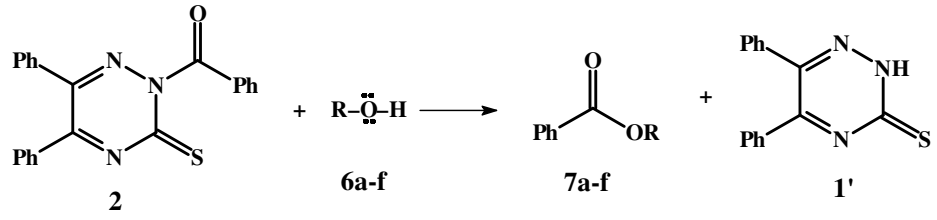


2' **3a-p** **4a-p** **1'**

R = **a**: C_6H_5- **b**: *o*-$C_6H_4(CH_3)$- **c**: *m*-$C_6H_4(CH_3)$-
d: *p*-$C_6H_4(CH_3)$- **e**: *p*-$C_6H_4(Cl)$- **f**: *p*-$C_6H_4(OCH_3)$-
g: C_6H_5-CH_2- **h**: N-$C_6H_5(C_2H_5)$- **i**: CH_3-
j: CH_3-CH_2- **k**: CH_3-CH_2-CH_2- **l**: CH_3-CH_2-CH_2-CH_2-
m: HO-CH_2-CH_2- **n**: (HO-CH_2-CH_2-)$_2$ **o**: HO-CH_2-CH_2-CH_2-CH_2-
p: *p*-$C_6H_4(OH)$-

Scheme-II

Acyl halide or anhydride method, therefore, is applied to make the process essentially irreversible. Transesterification in the presence of acidic catalysts such as *p*-toluene sulphonic acid is another pathway for the synthesis of esters. The smooth benzoylation of amines using N-BzDTT (**2'**) under room temperature conditions prompted to extend this nucleophilic reaction with other weak nucleophiles such as alcohols. Thus, when **2'** in chloroform was added to an equimolar quantity of ethyl alcohol, even after stirring for a long time, no evidence for the regeneration of 3-MDT was noticed. But, when the reaction mixture was heated to about 70 ºC for 5 min, the solution turned pale red orange with a clear fruity smell of the ester. Column chromatographic separation yielded ethyl benzoate and 3-MDT was regenerated in almost quantitative yield. Further, the very slow regeneration of **2** was also monitored spectrophotometrically. To generalize the esterification, the reaction was tried with other alcohols (**Scheme-III**). The above observations clearly demonstrate that though alcohols can be used as nucleophiles, they are weak in nucleophilic character to attack the already activated acyl group present in N-BzDTT (**2'**). It was also observed that N-BzDTT (**2'**) does not react with water.



2 **6a-f** **7a-f** **1'**

2 **6a-f** **7a-f** **1'**

R: **a**: CH_3- **b**: CH_3-CH_2- **c**: CH_3-CH_2-CH_2- **d**: CH_3-CH_2-CH_2-CH_2-
e: CH_3-CH_2-CH_2-CH_2- **f**: C_6H_5-CH_2-

Scheme-III

The above aminolysis and esterification reactions clearly show that the reactivity of N-BzDTT (**2'**) is sufficient enough to couple directly with the amino group under room temperature conditions and alcoholic group requires higher temperaure due to the weak nucleophilic nature of alcohols. The analytical details of aminolysis and esterification are presented in Table-1.

Reactions of N-BzDTT (2') with amino alcohols (selective aminolysis): The facile aminolysis with amines under room temperature conditions and the sluggish nature of esterification using alcohols prompted to carry out the selective aminolysis using amino alcohols. In the case of amino alcohols, amino nucleophiles were selectively reacted and the respective amides were obtained in good yield.

Thus, a chloroform solution of N-BzDTT (**2'**) when treated with an equimolar solution of ethanolamine in chloroform, a pale yellow solid was obtained. The reaction mixture was further stirred for 5 min. TLC and spectrophotometric investigation showed the formation of 3-MDT (**1**). The solid product obtained was separated using neutral alumina column. One fraction separated in 75 % yield was identified as N-(2-hydroxyethyl)benzamide m.p. 161 ºC (lit.[20] m.p. 163 ºC). The selective aminolysis was also repeated using diethanolamine and *p*-aminophenol. Here also 3-MDT (**1**) was regenerated which was identified by the colour change as well as spectrophotometrically.

Spectrophotometric monitoring of aminolysis and esterification using N-BzDTT (2'): The carbonyl and thiocarbonyl chromophores exhibit qualitatively similar bonding properties since both possess similar nuclear frame work. The $n \rightarrow \pi^*$ transition of C=S chromophore is found at longer wavelengths than the corresponding carbonyl compounds. This is understandable since the ionization potential of the sulphur lone pair is smaller than that of the oxygen lone pair. These differences in the energy and spacing of electronic states lead to differences in the absorption spectra of the compounds. These electronic absorption spectral characteristics of thiocarbonyl chomophores facilitated the study of the rates of aminolysis and esterification reactions using N-BzDTT. Further, in order to monitor the aminolysis and esterification spectrophotometrically, the rate of regeneration of 3-MDT (**1**) from N-BzDTT (**2'**) during the course of aminolysis/esterification reactions was followed since 3-MDT and N-BzDTT give distinct peaks differing in absorbance in their UV-visible spectra.

Thus, a qualitative study of the UV-visible absorption spectra of 3-MDT (**1**) and N-BzDTT (**2'**) during aminolysis and esterification reactions was carried out to correlate these with the extent of the reactions. These experimentations are carried out by observing the intensity of the absorption bands at specific wavelengths at regular intervals. Since the aminolysis reactions taking place are very fast in nature, only qualitative correlation can be made here.

Here a dilute solution of aniline (0.1 mmol) was mixed with an equimolar solution of N-BzDTT (**2'**) in chloroform and the course of aminolysis reaction was followed by spectrophotometric scannings at every 30 s. It was found that the initial

absorbance value at 1.7 gradually increased and after 5 min it remained constant. It is obvious from the relative absorbance values that, as the reaction proceeds, more and more 3-MDT is regenerated so that absorbance value gradually increased. Initial sudden jump in the absorbance shows that the major portion of the aminolysis would have completed within 2-4 min. The gradual increase in the absorbance after the above period is a clear indication of the slow process. The clear demonstration of the aminolysis reaction as a spectrophotometric tool paved the way to extend the reaction using different aliphatic and aromatic primary amines and also selective aminolysis with amino alcohols. Thus, the spectrophotometric scannings were repeated using *o*-toluidine, *n*-propylamine, *n*-butylamine. In all spectral scannings the regeneration of DTT (**2'**) is in conformity with the nucleophilicity of NH_2 group or OH group present. From the experimental observations, it is obvious that the esterification rections of N-BzDTT (**2'**) is sluggish at room temperature. As already evidenced, the weak nucleophilic nature of the alcohols and phenols were also established by the spectrophotometric studies on the esterification reactions using N-BzDTT (**2'**) with different alcohols like ethanol, benzyl alcohol, *m*-nitrophenol and *m*-cresol. Thus, when a chloroform solution of benzyl alcohol (0.1 mmol) was added to an equimolar solution of N-BzDTT (**2'**) followed by spectral scanning at definite time intervals of 30 s, only a very slow increase in the absorption of the characteristic peak at 321 nm was observed, which shows that the regeneration of DTT *i.e.* rate of esterification is very slow at ambient temperature.

Conclusion

N-Benzoyl-5,6-diphenyl-1,2,4-triazine-3-thione (**2'**) is prepared by the Schotten Bauman method and also by the DCC coupling using benzoic acid in order to establish the carboxyl activation ability of 3-mercapto-5,6-diphenyl-1,2,4-triazine. This is proved chemically by simple aminolysis and esterification of amines and alcohols respectively at ambient temperature conditions UV-visible absorption spectra of NBzDTT obtained during aminolysis and esterification function confirms the facile carboxyl activation capability of 3-mercapto-5,6-diphenyl-1,2,4-triazine.

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