J. Biol. Chem. Chron. **2020, 6**(*2*), 16-21
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 – 7476
www.eresearchco.com/jbcc/

Synthesis of 3-Substituted benzyl-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine and Assignment of Structural Formula

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DOI: http://dx.doi.org/10.33980/jbcc.2020.v06i02.003

(Received 29 Jul, 2020; Accepted 26 Sep, 2020; Published 01 Oct, 2020)

ABSTRACT: A variety of reaction in which hydrazides undergoes and gives the different products which depend upon the nature of hydrazide. Substituted phenyl acetic acid was prepared from corresponding substituted benzaldehyde by azlactone synthesis or by other means. The substituted phenyl acetic acid was converted into their hydrazides by standard procedure reacted with benzoylchloride and the product was heated with phosphorous oxychloride, product was treated with hydrazine under a variety of conditions to furnish unknown 3-substituted benzyl-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine. The structure of tetrazine thus prepared was confirmed by carrying out their synthesis in an alternative method. In alternative manner this consisted of converting the substituted phenyl acetic acid in its corresponding acid chloride reacting it with hydrazine of other benzoic acid followed by treatment with phosphorous oxy-chloride and then cyclizing the resulting product with hydrazine at the same tetrazine. 3-substituted benzyl-6-phenyl-1,2-dihydro1,2,4,5-tetrazine synthesized have been adequately characterized by elemental analysis, melting point and mixed melting point, IR and NMR data.

Keywords: Hydrazides; p-bromobenzaldehyde; benzoylchloride; phosphorous oxychloride.

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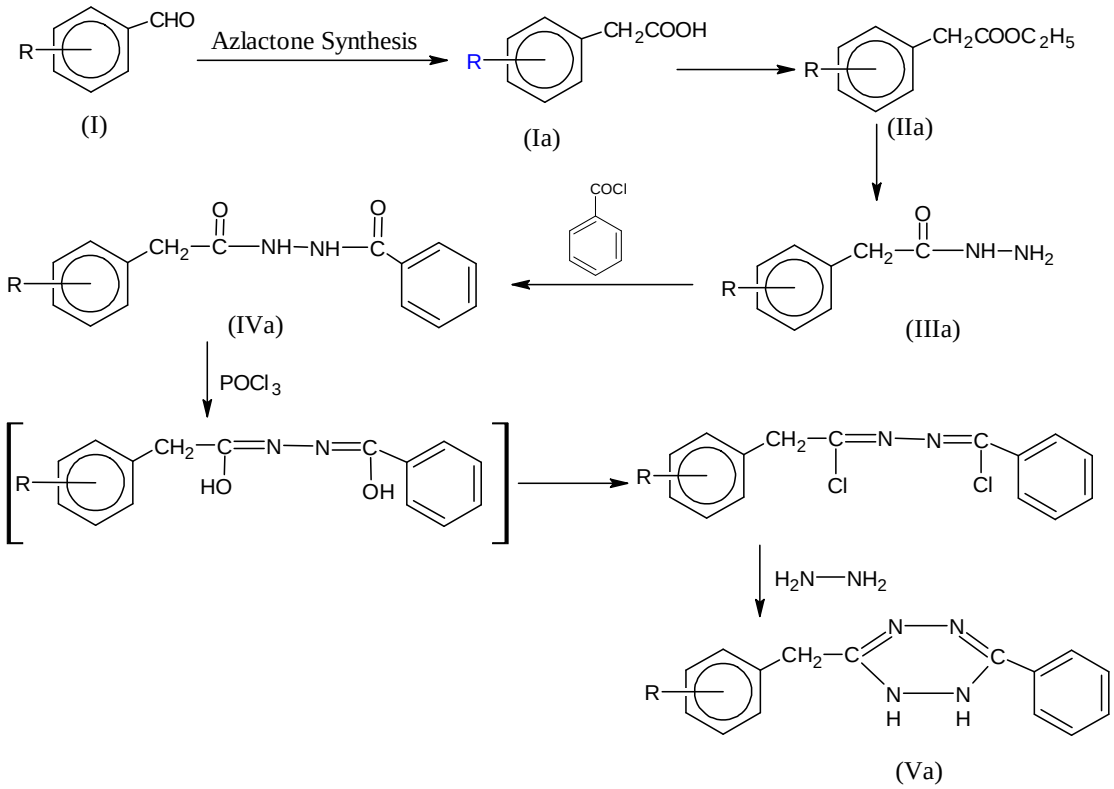
INTRODUCTION: Substituted tetrazines play important role in medicinal field. Tetrazine possess a wide range of antiviral and antitumor properties and have also been used as pesticides and herbicides.[1] These compounds are pharmacologically active, which have been extensively studied during past two decades. Various routes[2-10] have been reported in literature for synthesis of different substituted-1,2,4,5-tetrazine. Few of them[11-13] are for synthesis of 3,6-symmetrically disubstituted tetrazine obtained from the treatment of nitrile derivatives with hydrazine or aldehyde with substituted hydrazines.

Some 3,6-unsymmetrical disubstituted-1,2,4,5-tetrazines are rare heteroarene and biological active molecules[14]. Recently new substituted-1,2,4,5-tetrazine have been successfully synthesized by N-aryl isocynodichloride.[15-16] Such significant and diversified pharmaceutical values of substituted tetrazines have attracted our interest on the studies towards the search of new route for the synthesis of 3,6-disubstituted tetrazine.

In this study we wish to explore the route for the synthesis of 3,6-disubstituted tetrazine molecule by using p-bromo benzaldehyde[16] as one of the reagent for the synthesis of heterocyclic system and synthesize the molecule 3-p-bromo benzyl-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine.When compared with other classical methods, our procedure has advantages of simple operation, short reaction time, high yield, easily work-up and environmental benefits over the reported methods. It is a facile and convenient method.

To our knowledge, such process has not been previously demonstrated.

The structure of the proposed compounds are confirmed by I.R, NMR, Mass spectrum elemental analysis and finally with alternative method of synthesis.



Ia, IIa, IIIa, IVa, Va *where R = p-Br*
Ib, IIb, IIIb, IVb, Vb *where R = p-NO2*
Ic, IIc, IIIc, IVc, Vc *where R = o-Br*
Id, IId, IIId, IVd, Vd *where R = m-Br*
Ie, IIe, IIIe, IVe, Ve *where R=p-OCH_3*
If, IIf, IIIf, IVf, Vf *where R = m-OCH_3*
Ig, IIg,IIIg, IVg, Vg *where R = o-CH_3*

Figure 1: Formation of 3-(substituted benzyl)-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine by proposed method.

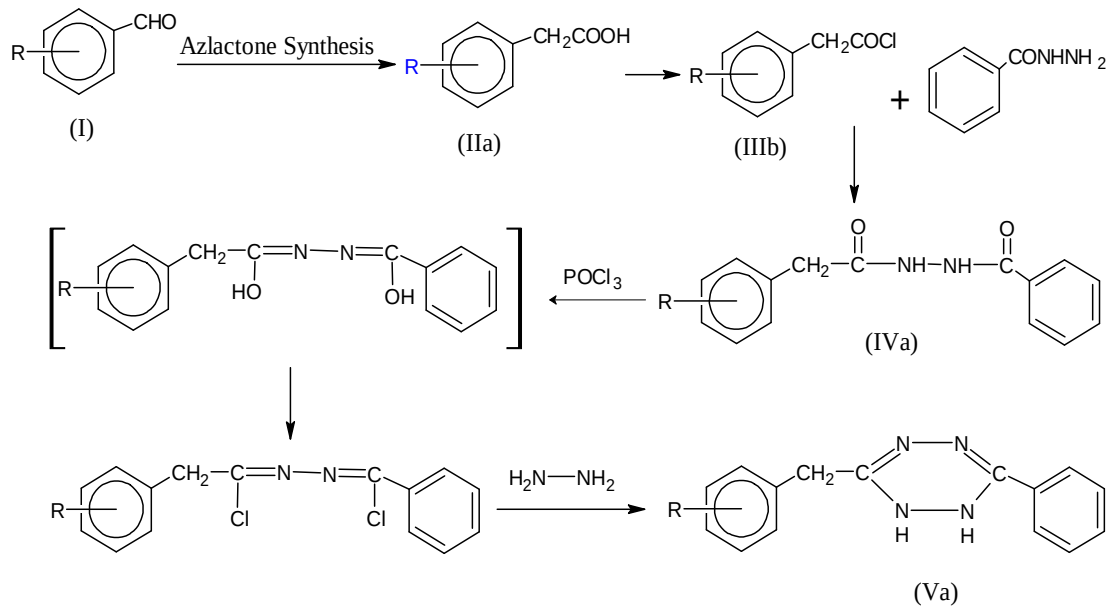


Figure 2: Formation of 3-substituted benzyl-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine by alternative method.

MATERIALS AND METHOD: All melting points are uncorrected and were obtained in capillary using paraffin both FT-IR spectra were recorded using KBr disc on Perkin-Elmer FT-IR Spectrophotometer and 'HNMR on Brucker advance 400.

NMR spectrometer using DMSO, $CDCl_3$ as solvent. Purity of the compound is checked on silica gel G plate using iodine vapour as a visualizing agent. All aryl substituted compounds were prepared by the extension of the known procedure.[16]

Preparation of substituted phenyl acetic acid: This was prepared from substituted benzaldehyde by azolactone synthesis.

A mixture of substituted benzaldehyde (15.4g), and hippuric acid (12g), acetic anhydride (18ml) and anhydrous sodium acetate (6g) was heated under reflux when mixture became homogenous in about half an hour. The resulting solution was boiled for a further one hour, cooled and left in an ice-chest overnight. The precipitated yellow solid was removed by filtration, washed several times with cold water and finally with boiling water.

The azlactone obtained above was heated under reflux with a solution of sodium hydroxide (NaOH) (25gm in 100ml of H_2O) for one hour. The reaction was then cooled, diluted with water (150ml) and then saturated with Sulphur dioxide. The reaction mixture was then filtered. The filtrate was heated on steam bath in an open dish with concentrated hydrochloric acid (75ml) and then cooled.

The precipitated substituted phenyl pyruvic acid was collected and crystallised from benzene. The crystals were collected by filtration and heated under reflux in a around bottomed flask with excess of hydrogen peroxide. The reaction mixture was cooled, and separated solid was collected by filtration. It was washed with cold water and recrystallized form hot water to give crystals of substituted phenyl acetic acid (12.6g), m.p. 113°C. Elemental analysis of this compound is given in Table 1.

Preparation of ethyl substituted phenyl acetate: A mixture of substituted phenyl acetic acid (12g), absolute ethanol (20ml) and concentrated H_2SO_4 (1ml) was heated under reflux on the water bath for two hours and then left at room temperature and left overnight. The excess of ethanol was then removed by distillation and the residual liquid washed with water, aqueous sodium bicarbonate solution (10%) and again several times with water. The liquid was dried over exiccated magnesium sulphate, distilled at 147°C under reduced pressure (18-20 mm) to give ethyl-substituted phenyl acetate as a colourless liquid (11.6g) (b.p. 142-144°C /12-14 mm) m.p.- 30°C. Elemental analysis of this compound is given in Table 1.

Preparation of substituted phenyacethydrazide: Following the method of Vogel, ethyl substituted phenyl acetate (11g) in ethanol (30ml) was heated with hydrazine hydrate (5ml, 85%) under reflux for three hours, when homogenous solution was obtained. The volume of solution was reduced to half. On cooling this solution, white solid is separated which was collected by filtration. Recrystallization of solid from methanol furnished substituted phenyl acethydrazide (9.5g) as white crystals m.p.- 193-194°C.Elemental analysis of this compound is given in Table 1.

Preparation of N-benzoyl substituted phenyl acethydrazide: A mixture of substituted phenyl acethydrazide (0.5g), benzoyl chloride (0.4ml) and NaOH solution was taken in small round bottomed flask and heated gently on water bath with occasional shaking. The reaction was followed by TLC measurement which should the complete disappearance of starting materials after one hour of heating eliminating hydrogen chloride gas. The residue on cooling deposited yellowish coloured solid. This was washed with benzene and ethanol. It was then recrystallsed from ethanol to furnish the pure N-benzoyl substituted phenyl acethydrazidem.p.- 167°C. Elemental analysis of this compound is given in Table 1.

Preparation of 3-(substituted benzyl)-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine: (By reaction between N-benzoyl- substituted phenyl acethydrazide with $POCl_3$ and NH_2-NH_2. The condensation was done under variety of conditions).

A mixture of N-benzoyl substituted phenylacethydrazide (0.5g) hydrazine hydrate (0.4g 85%), phosphorous oxychloride (1g) and carbon tetrachloride (0.5ml) was heated under reflux on water bath for about four hours. TLC examination showed that the reaction was complete. The reaction mixture was then left in an ice-chest overnight. Next day ash coloured solid which separated from reaction mixture was washed with benzene and then with alcohol. It was then recrystallized from ethanol to furnish the pure 3-substituted benzyl-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine a ash coloured needles (0.54g). Elemental analysis of this compound and spectral datas confirmed its identify. IR : C=C 1585cm^{-1}, Aromatic-Br 1510cm^{-1}, C-H 2920cm^{-1} indicating benzylic group, NH-NH bend. 1450cm^{-1}, C-N bend 1400cm^{-1} ^{1}HNMR: 2H Singlet at 8.5 δ heterocyclic ring proton. Mass: (m^+/e)-ratio.

Alternative Method of Preparation: ***Preparation of substituted phenylacetylchloride:*** In alternative method the above prepared substituted phenyl acetic acids were converted into their corresponding acid chlorides. These acid chlorides were treated with benzhydrazide resulting in the formation of N,N-diacylhydrazides, which were forced to eyclise under treatment with phosphorous oxychloride and 85% hydrazine. This produced the same 3-substituted benzyl-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine, which have been adequately characterized by elemental analysis, melting point mixed melting point and comparing the spectral data obtained in both the methods.

Table 1: Physical datas, (In point, yield) & mass spectra of compound (Va - Vg).

	M.P/B.P	C°/O	H°/O	N°/O	O°/O	Br	(m/e)⁺
$C_{15}H_{13}N_4Br$ P-bromo	195°C	54.70 (54.71)	3.92 (3.951)	17.00 (17.021)	-	24.25 (24.315)	328.251
$C_{15}H_{13}N_4Br$ O-bromo	156°C	54.65	3.90	16.98	-	24.20	328,251
$C_{15}H_{13}N_4Br$ M-bromo	118°C	54.58	3.90	16.98	-	24.20	328,251
$C_{16}H_{16}N_4O$ P-methoxy	162	68.50 (68.517)	5.50 (5.71)	19.95 (20)	5.70 (5.717)	-	279.262
$C_{16}H_{16}N_4O$ O-methoxy	142	68.55	5.55	19.90	5.62	-	279,262
$C_{16}H_{16}N_4O$ M-methoxy	224	(68.52)	5.60	19.92	5.68	-	279,262
$C_{15}H_{13}N_5O_2$ P-Nitro	172°C	60.95 (61.0169)	4.25 (4.406)	23.70 (23.728)	10.80 (10.847)	-	294,217
$C_{15}H_{13}N_5O_2$ O-Nitro	117°C	60.90	4.20	23.65	10.75	-	294,217
$C_{15}H_{13}N_5O_2$ M-Nitro	186°C	60.92	4.30	23.60	10.82	-	294,217

RESULTS AND DISCUSSION: In the tetrazine formation, the required cyclisation was affected by heating the reactants with $POCl_3$ in pyridine for four hours and also heating the reactants directly for several hours. The progress of reaction was checked by TLC examination form time to time. The yield of the cyclized product in all cases was fairly good. Since acidic reagents were found to be more effective at the same time basic reagents were also found good enough in bringing about such cyclization. The result had a sharp melting point and was shown by TLC examination to be a pure compound. The reaction scheme of the method of synthesis of 3-substituted benzyl-6-phenyl-1,2-dihydro-1,2,4,5-tetrazine The IR spectra of this compound displayed N-H stretching band of tetrazine ring. C-H stretching vibration, NHNH bending vibration and C-N bending vibration were observed at 1585 cm^{-1}, 1450 cm^{-1} and 1400 cm^{-1} respectively substituted aromatic of benzene ring showed a band at 1610 cm-1. C-H stretching bands indicating benzylic group of molecule were observed at 2920 cm^{-1}. The ^{1}HNMR spectrum of compound indicated that signal of two sets of NH protons were observed one singlet at 6.75 δ. The Mass spectrum of compound showed presence of peak at m+ /e 329. Elemental analysis of all the intermediates and resulting product obtained by method of synthesis indicated the molecule formula and finally structural formula. On the basis of above facts and evidences the compound was assigned the structure of 3-substituted benzyl 6- phenyl-1,2-dihydro-1,2,4,5-tetrazine. Results and Discussions For the synthesis of compounds (Ia to IVa), (Ib to IVb), (Ic to IVc), (Id to IVd), (Ie to IVe), (If to IVf) and (Ig to IV g) standard methods have been employed. The proposed products of 3,6-disubstituted,2-dihydro-1,2,4,5-tetrazines (Va,Vb,Vc Vd,Ve,Vf and Vg) were prepared by using heating directly and in the solvents carbon tetrachloride, pyriding under high heating as well as room temperature both. The formation of the product was checked time to time by monitoring the thin layer chromatography. Infrared spectrum of compounds (IVa -IV g) showed the presence of –C= O bonds, while IR bands of compound (Va to Vg) indicated the absence of bond –C=O . In IR spectra do not show the band for stretching frequency of the hydroxyl (or chloro) confirming the reaction undergoes in suitable manner to form a tetrazine ring. The IR spectrum displayed the band due to V_{NH}, $V_{C=N}$ $V_{C=N-N=C}$ V_{NH-NH} groupings for $V_{NH-C=o}$ -3300 cm^{-1}, $V_{C=N}$ = 1580 cm^{-1}, $V_{C=N-N=C}$ - 1700 cm^{-1}, V_{NH-NH} =1560 cm^{-1} substituted benzene ring - 1670 cm^{-1} and 2950 cm^{-1} for -C-H

stretching. The pmr spectrum of the compound indicated the signals of -NH-NH-protons, methylene (-CH_2-) protons and aromatic protons as shown below.

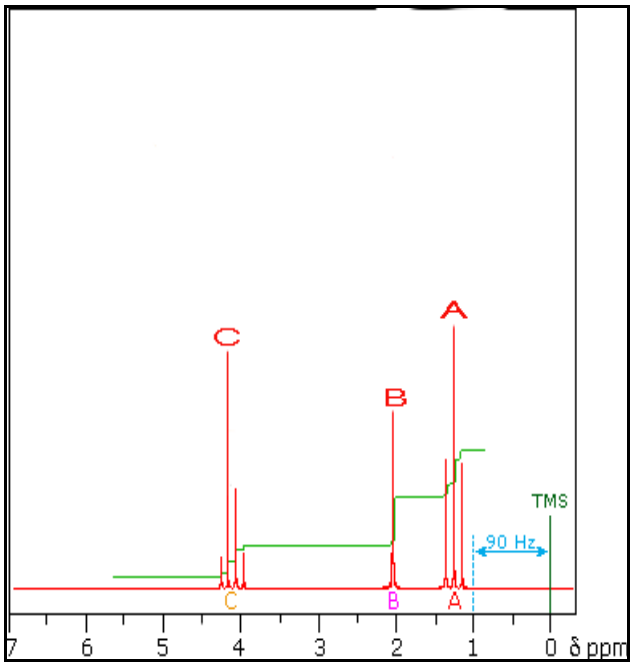


Figure 3 : pmr spectrum of 3-(substitutedbenzyl)-6-phenyl-1,2-dehydro-1,2,4,5-tetrazine.

In Figure 3, 'HNMR A singlet at 1.75δ equivalent to 2 H indicating the two identical H (for-NH-NH-) protons (a) A quarlet at 4.06 δ equivalent to 2 H indicates the methylene protons (b) A multiplet resonauce for aromate protons 7.4-8.2 δ (c) The mass spectrum of the compound showed presence of (M-1)+ peak at m/e = 328, other important fragment peaks were located at 256, 180, 166 etc in the product Va. The elemental analysis of the product Va indicated the molecular formula $C_{15}H_{13}N_4$ Br . On the basis of all the above facts the compound Va was assigned structure as 3-(substitutedbenzyl)-6-phenyl-1,2-dehydro-1,2,4,5-tetrazine. The structure of compound Va was also confirmed by alternative method of synthesis. Compounds obtained in each and every step using standard method, were compared with the compounds obtained in previous method it was found that elemental analysis, melting point & mixed melting points are same: More over the comparison of data & signals obtained in their spectral analysis e.g IR, 'HNMR, Mass are also identical This confirmed the identity of same structure as proposed above for the substitued 1,2-dehydro tetrazines. On the basis of all compounds (Ib to Vb), (Ic to Vc), (Id to Vd), (Ie to Ve), (If to Vf) and (Ig to V g) were prepared and the structure of Vb, Vc, Vd, Ve, Vf & Vg were assigned and successfully confirmed structures as below in figure 4

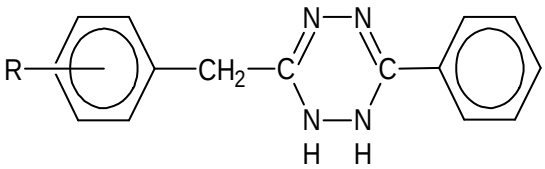


Figure 4: 3-substituted benzyl-6-phenyl-1,2-dihydro-1,2,4, 5-tetrazine.

CONCLUSION: Here we have explored the route for the synthesis of 1,2-dihydro-1,2,4,5-tetrazines by using diaroylhydrazides & hydrazine reapent for the synthesis of heterocycle system.

It is concluded from the result and discussions that 'Route method and alternative method' can be used for the synthesis of 3-substituted benzyl-6-phenyl-1,2-dihydro-1,2,4, 5-tetrazine.

ACKNOWLEDGEMENT: The authors are very thankful to University Department of Chemistry, L.N.M.U ,Darbhanga and also to Prof. S. R. Kumar and Prof. K. K. Jha who co-operated in their research lab for programming works as well as providing concept and suggestions for completion of any work.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interests regarding the publication of article.

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