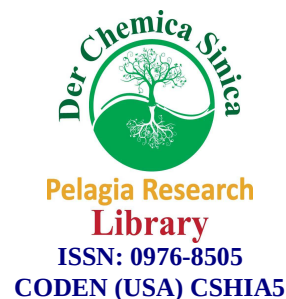




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Synthesis and bioelectrochemical screening of modified Tolbutamide molecule

Rakesh Choure*, Neelam Vaidya and K.S. Pitre

Department of Chemistry, Dr. Hari Singh Gour University, Sagar(M.P.), India

ABSTRACT

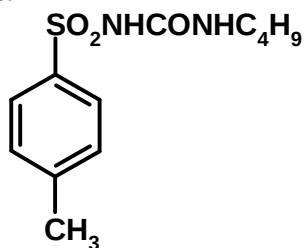
Tolbutamide is an antidiabetic drug. Its potency may be increased by modifying the drug by way of molecular modification. In the present study the drug has been modified by its complex formation with potassium bicarbonate and other organic compounds. The drug organic compound interaction has been studied using differential pulse polarography and direct current polarography. In KCl as supporting electrolyte of $pH\ 3.4 \pm 0.1$, tolbutamide produced a well defined peak at $-1.02v$ and its modified forms at relatively higher negative. The change in peak potential and lowering in peak height. Drug organic compound complexation has completed.

Keywords: Tolbutamide, DCP, DPP, glucometer, diabetic activity etc.

INTRODUCTION

Anti-diabetic drugs treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents. Sulfonylureas were discovered by the chemist Marcel Janbon and co-workers,[1] who were studying sulfonamide antibiotics and discovered that the compound sulfonylurea induced hypoglycemia in animals.⁽²⁾ Research has shown the Maitake mushroom (*Grifola frondosa*) has a hypoglycemic effect, and may be beneficial for the management of diabetes.[3-7] The reason Maitake lowers blood sugar is due to the fact the mushroom naturally acts as an alpha glucosidase inhibitor. Other mushrooms like Reishi,[8,9] *Agaricus blazei*,[10-13] *Agrocybe cylindracea*[14] and *Cordyceps*[15-19] have been reported to lower blood sugar levels to a certain extent, although the mechanism is currently unknown Walnut leaf can significantly reduce fasting blood glucose levels in rats with alloxan-induced diabetes, and rats thus treated show some evidence of regeneration of the beta cells.[20] Garlic also significantly reduces fasting blood glucose levels in rats with alloxan-induced diabetes.[21] Tolbutamide $C_{12}H_{18}N_2O_3S$, is a first generation potassium

channel blocker, sulfonylurea oral hypoglycemic drug [22]. It is a white crystalline substance, used to augment insulin secretion in the treatment of diabetes mellitus [23, 24]. This drug may be used in the management of type II diabetes if diet alone is not effective. Tolbutamide stimulates the secretion of insulin by the pancreas [25]. Since the pancreas must synthesize insulin in order for this drug to work, it is not effective in the management of type I diabetes. It is not routinely used due to a higher incidence of adverse effects compared to newer second generation sulfonylureas [26], such as glyburide.



Tolbutamide

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used to prepare experimental sets were of Sigma Aldrich/Loba chemical grade. KCl from Loba chemical grade was used. Solutions of the reagents were prepared by dissolving a requisite amount of the respective chemical in double distilled water.

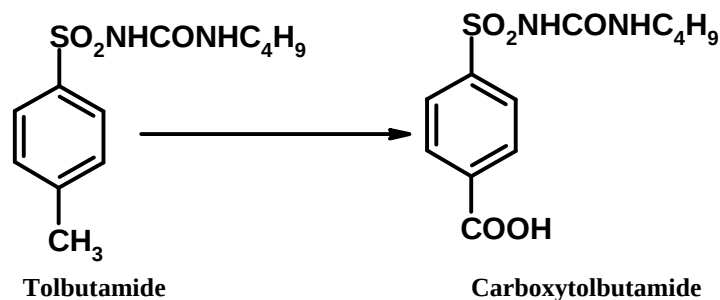
The drug, under study was procured from the market, i.e. Tolbutamide [Sigma Aldrich Ltd.]. The stock solution of the drug was prepared by the following method:

Tolbutamide: ($\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$, White powdered compound, M.W. - 270.35)

0.01M stock solution of tolbutamide was prepared by dissolving the weighed amount of the drug in water and alcohol.(3/2 v/v).

Synthesis and analysis of Carboxytolbutamide

Tolbutamide molecule was modified following the procedure discussed below. Tolbutamide (5gm) was added to, potassium bicarbonate (25gm) and water (50ml), the mixture was heated for four hours in a steam bath in a three necked round bottom flask (500ml capacity) fitted with a reflux condenser and a gas inlet tube. It was then refluxed for 30 minutes while passing a rapid stream of carbon dioxide gas through the solution. The solution was acidified while hot by adding concentrated hydrochloric acid (20ml) from a separatory funnel with a long tube delivering the acid to the bottom of the flask. The flask was allowed to cool to room temperature and then kept in an ice bath. The separated part was filtered and crystallized with hot water. The white crystalline solid was carboxytolbutamide. The yield of carboxytolbutamide was 2.7gm.



Preparation of analyte and recording of the Polarogram

For polarographic study the experimental set was prepared by taking 1ml of 0.01M sample solution and 10ml (1M) of KCl as supporting electrolyte in a polarographic cell and the total volume was made to 50ml with distilled water. The pH of the test solution was adjusted to 3.4 ± 0.1 with dil. NaOH/HCl solution. After deaeration of the analyte, polarogram was recorded.

Pharmacological study

The antidiabetic activity of tolbutamide was screened by performing *in vivo* experiments on albino rats (60-80gm).

All estimations of blood glucose were done on an electronic “Thyrocare’s Sugar Scan Glucometer” using “Thyrocare’s Sugar Scan” reagent strips. The pharmacological studies were done at the Pharmaceutical Science Department of Dr. Hari Singh Gour University, Sagar (M.P.).

Induction of Artificial Diabetes in Albino rats:- 3 sets each with 15 albino rats (each rat weighing about 60-80gm) were selected and their fasting blood glucose levels were measured on the Glucometer. They were then injected intravenously (on tail) with a solution of 4.5mg/100gm alloxan in normal saline. The albino rats were allowed to develop diabetes for about 15 days. At this stage this blood glucose level was again measured and checked whether it was higher than 180mg/dl. A glucose level higher than this indicated diabetic condition and the albino rats were ready for *in vivo* evaluation. The albino rats were allowed a free access to food and water.

RESULTS AND DISCUSSION

Polarographic Analysis:

The direct current polarogram (DCP) and differential pulse polarogram (DPP) of the authentic sample solution of tolbutamide in KCl (.2M) at pH 3.4 ± 0.1 produced one well-defined polarographic wave/peak with $E_{1/2}/E_p = -1.02/-1.04V$ vs SCE.

To ascertain as to whether the wave/peak is due to tolbutamide present in the solution, a known quantity of standard solution of tolbutamide was added to the analyte and polarogram was recorded under above experimental conditions, An increase in wave height of the polarogram due to tolbutamide was observed without any change in half wave/peak potential, thus confirming the presence of tolbutamide in the solution.

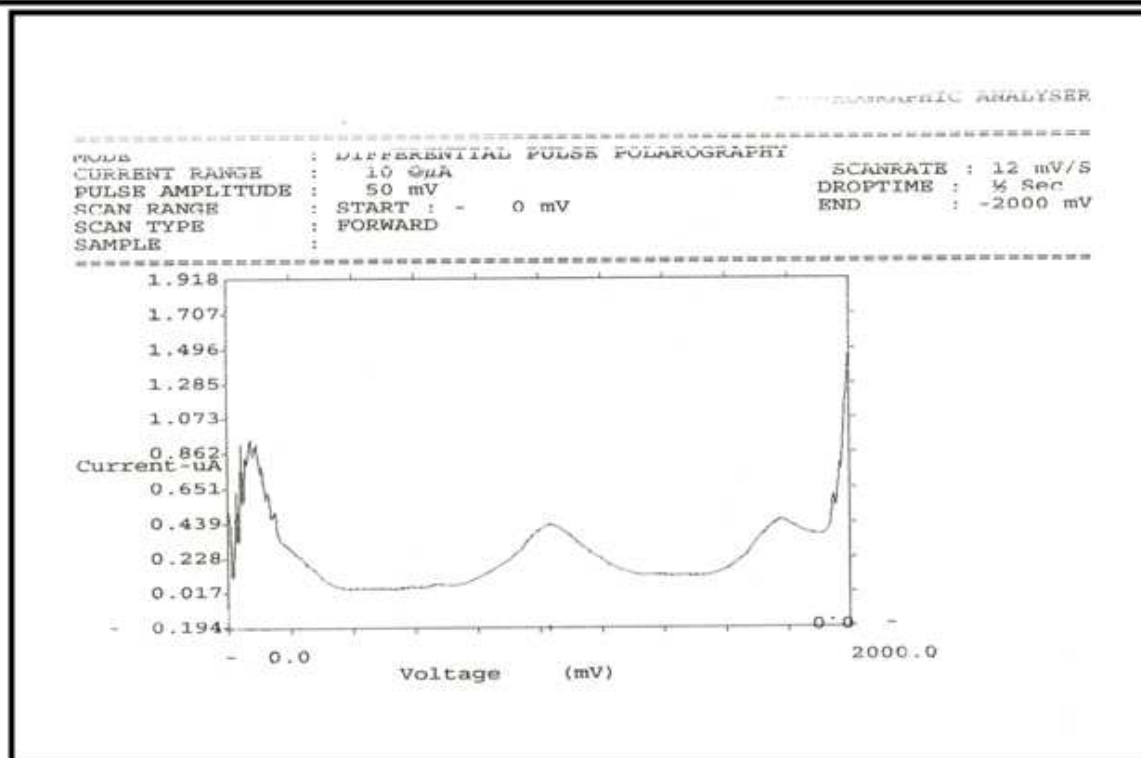
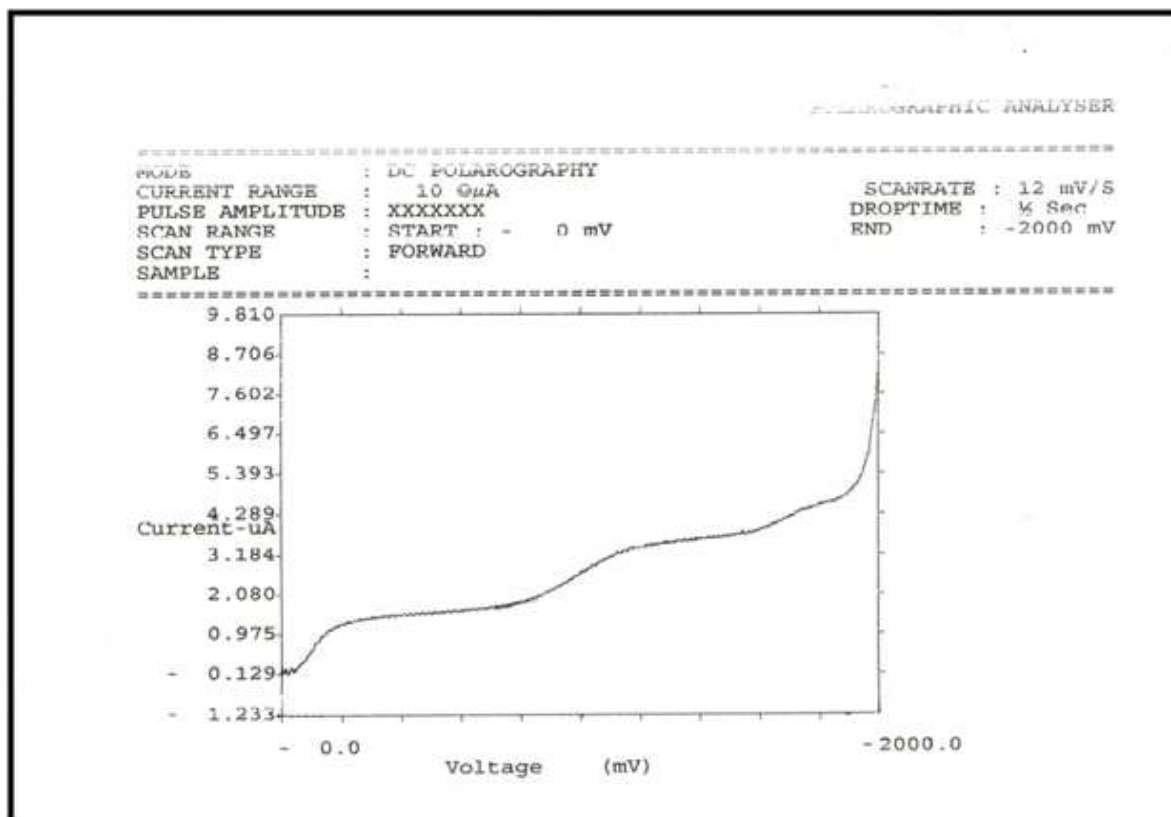


Fig.1 : Direct current Polarogram & Differential pulse Polarogram of (0.2mM) Tolbutamide in KCl (0.2M) pH 3.4 $\pm$ 0.1.

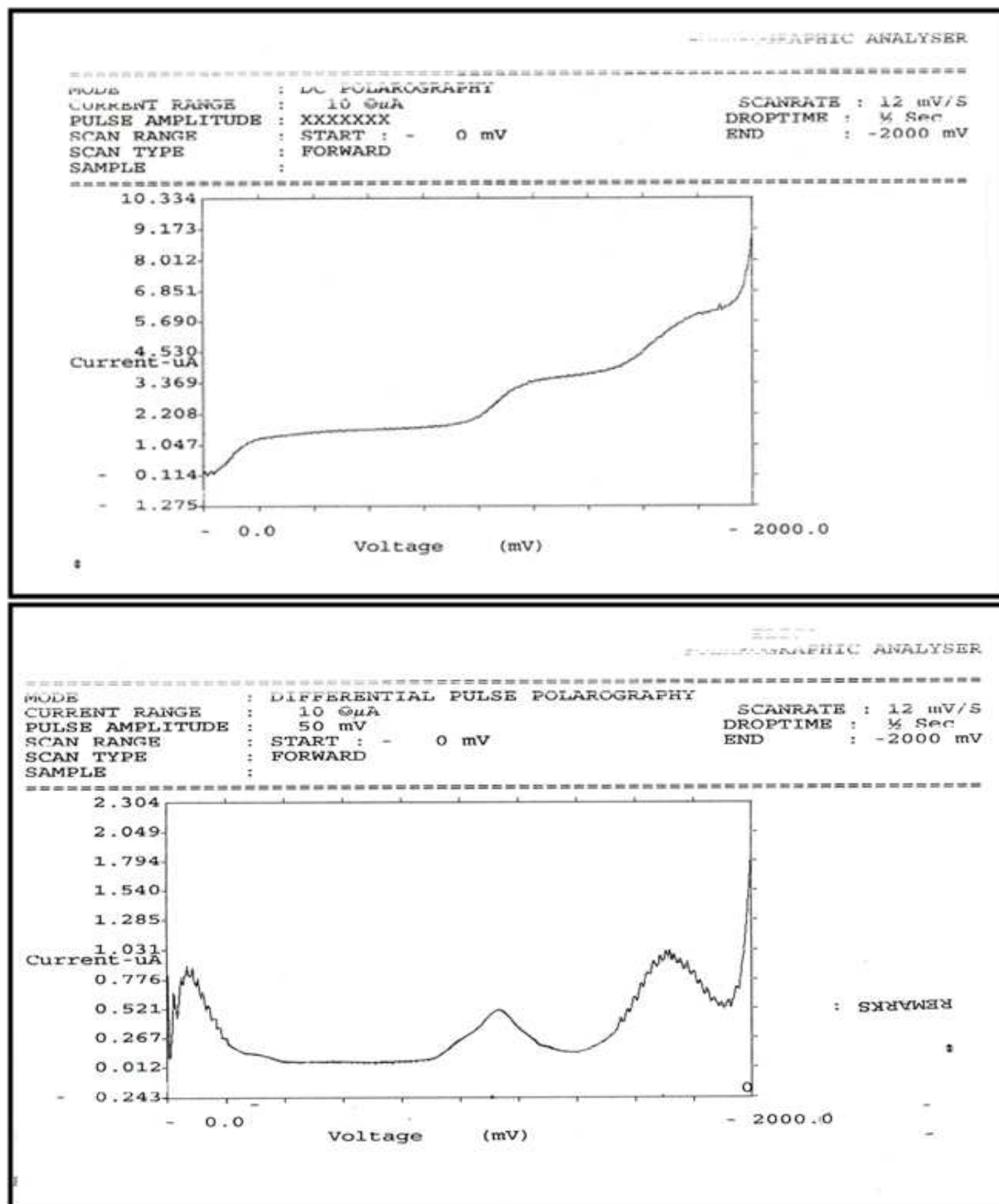


Fig 2 (a) Direct current polarogram & Differential pulse polarogram of (0.2mM) modified tolbutamide (Carboxytolbutamide) in KCl (0.2M) pH 3.4 $\pm$ 0.1.

On recording the polarogram of the modified drug (carboxytolbutamide) under similar experimental condition the resulting polarogram was also produced very well defined wave. The shift in half wave potential i.e. from -1.02V to -1.12V indicated the formation of carboxytolbutamide. The polarographic analysis of varying concentration of carboxytolbutamide under the above set experimental conditions also showed linear relationship between i_d and carboxytolbutamide concentration. Thus enabling its qualitative and quantitative analysis.

IR characterization of the test samples

The IUPAC name of tolbutamide is *N*-[(butylamino) carbonyl]-4-methylbenzenesulfonamide.

FTIR spectra of authentic tolbutamide (Fig. 4-1.3) clearly shows characteristic signals at 3410cm^{-1} (N-H), 1660cm^{-1} (-NH), 1381cm^{-1} (C_4H_9), 1725cm^{-1} (C=O), 1070cm^{-1} (SO_2), 1363cm^{-1} (methyl group), 1580cm^{-1} (aromatic ring).

On the other hand modified drug (Carboxytolbutamide) (Fig.3-1.4) has given characteristic signals at 3415cm^{-1} (N-H), 1660cm^{-1} (-NH), 1381cm^{-1} (C_4H_9), 1730cm^{-1} (C=O), 1065cm^{-1} (SO_2), 1701cm^{-1} (COOH), 1600cm^{-1} (aromatic ring). Thus confirming the presence of carboxylic group in modified form.

Pharmacological Experiments

The normal fasting glucose level of albino rats is in the range of 50-70 mg/dl. In case of normal albino rats the glucose level shot up immediately after administration of food. However, it came back to normal after the dose of tolbutamide respectively was given orally to the rat. The effect of the administered drug lasted only for about 10-12 hours.

When the experimental animals were given alloxan treatment intravenously. Their glucose level was in the range of 180-200 mg/dl up to twenty one days after the alloxan dose was given to them. Then an appropriate dose (0.036 gm/100gm) of the drug and its modified form were separately given orally to the rats. The blood sugar levels were then noted at different time intervals, the results have been tabulated in the following table [Table 4-1.1].

The table shows that the initial blood sugar level is 220 mg/dl which on administration of the tolbutamide drug and its modified form showed a decrease with time. It fell down to 96mg/dl in case of tolbutamide whereas the level observed was 85mg/dl in case of its modified form. Thus indicating that the modified form (carboxytolbutamide) is more potent as compared to tolbutamide.

Table- 1: Blood Glucose levels of diabetic albino rats after administration of Tolbutamide and its modified form

S.No.	Time interval (hrs.)	Initial blood glucose level (mg/dl)	Parent drug blood glucose level (mg/dl)	Modified drug blood glucose level (mg/dl)
1.	0	220	220	220
2.	2	-	210	208
3.	4	-	190	182
4.	6	-	175	162
5.	8	-	136	121
6.	10	-	113	103
7.	12	-	96	85

Amount of Tolbutamide and its modified form (Carboxytolbutamide), which were given orally to albino rats = 0.036gm/100 gm

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