SHORT COMMUNICATIONS

PINITOL—A NEW ANTI-DIABETIC COMPOUND FROM THE LEAVES OF *BOUGAINVILLEA SPECTABILIS*

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To confirm earlier reports[1] that the leaves of *Bougainvillea* can cure diabetes mellitus, we carried out experiments on the extract of the leaves. The leaves were repeatedly extracted with alcohol and then with water, finally leaving behind a cellulosic material. Only the alcoholic extract was active. It has been found that the alcoholic extract of *Bougainvillea spectabilis* (BVS) has significant hypoglycemic effect in normal as well as alloxan-induced diabetic albino mice and that it is free from any acute toxicity[2]. Raju[3] recently confirmed the hypoglycemic effect of the alcoholic extract of BVS.

The dried alcoholic extract of the leaves of the plant[2] was extracted successively by hot-percolation with petroleum ether, benzene, ethyl acetate and methanol. Preliminary testing on normal albino mice showed that the hypoglycemic action was concentrated in the methanol extract. The methanol extract was, therefore, chromatographed on silica gel, 60–120 mesh by successive elution with petroleum ether, benzene, ethyl acetate, their mixtures and finally with methanol. The methanol eluates gave the bulk of the material as a crystalline compound, M.P. 190°C $(\alpha)_D^{25}$ +65°, in about 10% yield of the total alcoholic extract.

This active crystalline compound has been characterized as D-chiro (+)-*o*-methyl inositol or pinitol, by its melting point, specific rotation, NMR, IR and mass spectral characteristics and also those of its derivatives[4]. This will be reported separately.

This compound has been tested for its hypoglycemic and anti-diabetic activity, in the same way as was reported for the total alcoholic extract[2]. A minimum oral dose of 0.01 g/kg was found to be significantly active. In all the three models used to screen the hypoglycemic and anti-diabetic action of pinitol, groups of 10 mice (each weighing 25–30 g) of either sex were used for each kind of treatment, that is for control and for experimental groups. In each model when the experimental group was treated with the drug (in water solution), the control group was given an equivalent amount of distilled water. Blood sample (0.2 ml) was taken from the jugular vein by sacrificing the animal and the blood sugar level (BSL) was estimated by the Folin Wu method[5].

(i) *Effect on blood sugar level of normal albino mice*

Pinitol was administered orally to normal albino mice fasted for 18 hr[6] at a dose level of 0.01 g/kg and their BSL was estimated after 1/2, 1, 2 and 4 hr intervals (table 1).

(ii) *Effect on alloxan-induced diabetes in albino mice*

Albino mice fasted for 18 hr were injected with alloxan (120 mg/kg) IP to induce diabetes[6]. After alloxan treatment, pinitol (0.01 g/kg) was administered orally at 24, 28, 48, 52 and 72 hr intervals. BSL of the control group was estimated at 0, 24 and 72 hr after alloxan treatment and the BSL of the experimental and control animals were estimated (table 2) 2 hr and 4 hr after 5 doses.

(iii) *Effect on glucose tolerance test (GTT)*

Albino mice were fasted for 18 hr. A group of mice was administered with pinitol 0.01 g/kg with appropriate controls. Glucose at the rate of 1.5 g/kg[7] was given orally to both the sets 30 min after the administration of pinitol. Then BSL of all the animals was estimated after intervals of 30, 60 and 90 min respectively (table 3).

It is evident from the tables that pinitol which is readily soluble in water, when administered orally at a dose level of 0.01 g/kg in normal fasted albino mice, was found to give significant ($P < 0.05$)[8] hypoglycemia, the maximum being at the end of 2 hr. In alloxan-induced diabetic mice, on chronic treatment of pinitol for 72 hr (5 doses), a significant fall in BSL was observed. The continuous decrease in BSL observed 4 hr after the administration of the last dose of pinitol shows that the hypoglycemic effect is persistent in the alloxan-induced diabetic mice. In case of glucose tolerance test, the same type of hypoglycemic effect was observed as with the total alcoholic extract[2]. All these results show that pinitol has significant hypoglycemic and anti-diabe-

Table 1 *Effect of pinitol on the blood sugar level (BSL) of fasting albino mice*

	Mean BSL in mg/100 ml ± S.E.				
	Initial 0 hr	after the administration of pinitol 1/2 hr	1 hr	2 hr	4 hr
Control	107.00 ±0.68	106.35 ±0.50	107.46 ±0.81	107.78 ±0.45	107.82 ±0.75
Treated with 0.01 g/kg of pinitol	107.75 ±0 76	96.75* ±0.64	94.38* ±0.49	84.62* ±0.86	108.50 ±0.62

* $P < 0.05$

Table 2 *Effect of pinitol on alloxan-induced diabetes in albino mice*

	Mean BSL in mg/100 ml ± S.E.				
	0 hr	24 hr	72 hr	74 hr	76 hr
Control only alloxan-treated (120 mg/kg)	110.00 ±0.68	198.43 ±1.76	267.75 ±1.40	270.47 ±1.23	272.78 ±1.57
Experimental Alloxan 120 mg/kg and one dose each of pinitol (0.01 g/kg) at 24, 28, 48, 52 and 72 hr after alloxan treatment	—	—	—	129.63* ±0.73	121.00* ±1.06

* $P < 0.05$

Table 3 *Effect of pinitol on glucose tolerance test*

	Mean BSL in mg/100 ml ± S.E.			
	0 hr	1/2 hr	60 min	90 min
Control				
1.5 gm/kg glucose	105 ±0.78	142.63 ±1.255	124.5 ±1.09	121.38 ±1.19
Experimental				
0.01 g/kg pinitol and after 1/2 hr 1.5 gm/kg glucose	100 ±1.15	121.47* ±0.78	110.23* ±1.14	102.54* ±1.19

* $P < 0.05$

tic action. The exact mechanism of action of pinitol requires further studies.

The common hypoglycemic or anti-diabetic drugs are sulphonamides, biguanides, substituted naphthylacetic acids and other allied synthetic compounds[9]. Oral hypoglycemic action has also been reported for natural products as hypoglycin A and B, charantin and some flavonoids[10]. Some weak hypoglycemic[11] and anti-diabetic[12] activity has been reported in the literature for inositol in general, along with other pharmacological activities[13] as potentiation of adrenaline secretion[14], cholesterol removal from blood[15] etc. But this is the first report of hypoglycemic as well as anti-diabetic activity of pinitol. This is also the first report of the isolation of pinitol, from the leaves of any *Bougainvillea* species.

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SYNTHESIS OF SOME NEW FLUORO-SUBSTITUTED NITROPHENOTHIAZINES

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THE zinc mercaptide of 2-amino benzene thiol and its substitutes have been used in the synthesis of a wide variety of phenothiazine compounds[1-3]. But little attention has been paid towards the synthesis of nitrophenothiazines especially by Smiles rearrangement[4]. With a view to explore the domain of such a reaction, we have studied the reaction of zinc mercaptide of 2-amino-4/5-fluorobenzenethiol with some reactive halonitrobenzenes in the presence of sodium hydroxide in absolute ethanol.

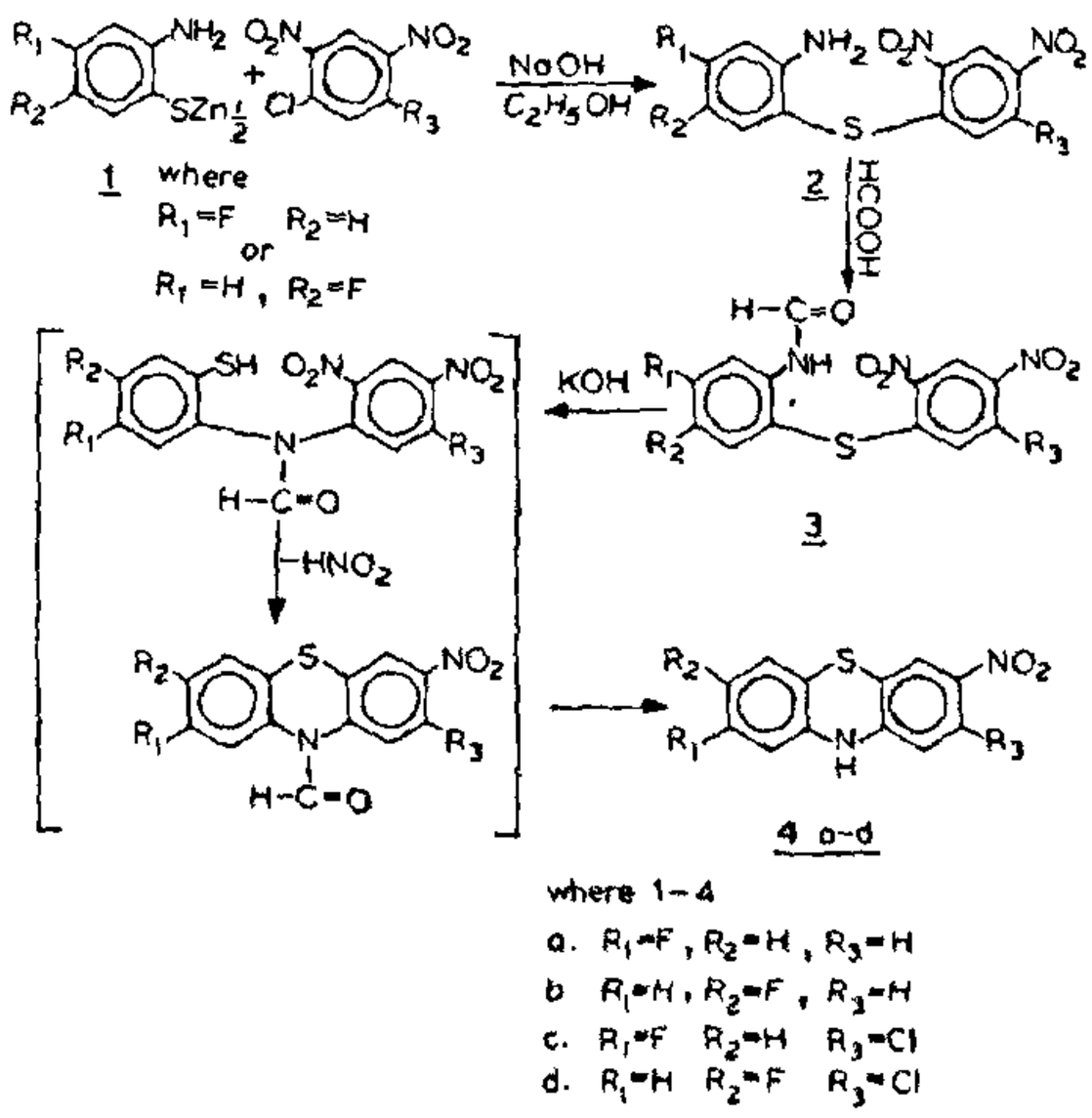
The experimental techniques were the same as reported earlier for 1-chloro-2,4-dinitrobenzene[5], 1,3-dichloro-4,6-dinitrobenzene[6], 1,2,3-trichloro-4,6-dinitrobenzene[7]. Zinc mercaptide of 2-amino-4/5-fluorobenzenethiol[8,9] were prepared by adopting the procedure already reported.

Preparation of nitrophenothiazines (4a-d, 5a-b, 6a-b, 7a-b)

To a refluxing mixture of zinc mercaptide of 2-amino-4/5-fluorobenzenethiol (1) (0.005 mol), sodiumhydroxide (0.01 mol) in absolute ethanol (20 ml) was added hot solution of reactive halonitrobenzene (0.01 mol) in ethanol (5 ml). The colour of the reaction mixture darkened immediately on the addition reactive halonitrobenzene. The refluxing was continued for 5–7 hr. After refluxing, the reaction mixture was cooled, filtered, washed well with hot water and finally with ice-cold dilute ethanol to obtain nitrophenothiazine.

Purification was effected by recrystallization from benzene to get a better sample of the title compound. The IR and mass spectral data of nitrophenothiazines (4a-d, 5a-b, 6a-b, 7a-b) are given in table 1. The R_f values of the compound (5a-b, 6a-b, 7a-b) are also reported and discussed. All the compounds synthesized are new and gave satisfactory elemental analysis (table 2).

We observed that 1-chloro-2,4-dinitrobenzene and 1,3-dichloro-4,6-dinitrobenzene reacts with zinc



Scheme-1