ISOLATION OF ORALLY EFFECTIVE HYPOGLYCEMIC COMPOUNDS FROM *FICUS BENGALENSIS* LINN.

By

H.D. BRAHMACHARI AND K.T. AUGUSTI

From the Department of Biochemistry, Birla College, Piliani

(*Received September 16, 1963*)

Three flavonoid Compounds A,B, and C have been isolated from the ethanolic extract of the bark of *F. bengalensis* Linn. Compounds A and C have been identified as different forms of some leucoanthocyanidin, while compound B proved to be a leucoanthocyanin (glycoside). All the three compounds individually were found to be effective as hypoglycemic agents on oral administration to normal fasting rabbits. Compound B showed the maximum hypoglycemic effect, out of these three compounds, when compared to tolbutamide. Compound B was also found to be fairly effective in controlling the hyperglycemia produced by the oral administration of glucose to normal fasting rabbits.

The presence of suitable orally effective hypoglycemic principles in the alcoholic extract of the bark of *F. bengalensis* Linn. has been reported earlier (Brahmachari and Augusti, 1961, 1962). The bark, leaves and fruits of this tree have been reported to contain mostly tannins and related compounds (Agri. Ledger, 1904; Sharma and Seshadri 1955). Deshmukh, Shrotri and Aiman (1960) reported the presence of a glycoside with hypoglycemic activity, in the ethanolic extract of the bark. They found however that the crude aqueous extract was more active than the pure glycoside. The isolation of some other compounds with hypoglycemic activity, from the active ethanolic extract, is described in this paper.

METHODS AND RESULTS.

The dry bark powder of *F. bengalensis* was extracted in a soxhlet with different grades of petroleum ether, diethyl ether, and 90/per cent ethanol successively. The active ethanolic extract, after the removal of alcohol under reduced pressure, was further extracted with ethyl acetate. The ethyl acetate soluble fraction on concentration, dehydration, and precipitation with petroleum ether (40°-60°C)., finally gave a colourless crystalline product (compound A) which melted at 218°C. The ethyl acetate insoluble portion was further extracted with water. The water soluble fraction on concentration, yielded a red mass which was extracted with alcohol and on addition of ethyl ether to the alcoholic solution, a buff coloured crystalline product (Compound B)

was obtained. It was recrystallised from 50 per cent alcohol. It melted at 210°C. By a similar procedure an amorphous red mass which darkened at 220°C (Compound C) was obtained from the water insoluble portion.

The solubility and colour reactions of the compounds A and C indicated that they were different forms of some leucoanthocyanidin. With alcoholic hydrochloric acid they developed a purple colour which deepened on warming. With alcoholic ferric chloride they gave a blue colour. The compounds A and C were converted into anthocyanidin chloride by the methods of Rosenheim (1920) and Robinson and Robinson (1933). The following tests were performed as suggested by Robinson and Robinson (1933) and Bate-Smith (1954) with the 1 per cent HCl solution of the anthocyanidin chloride obtained separately from A and CI.—

(1) 1 ml of the solution was treated with amyl alcohol (3 ml), and enough of solid sodium acetate to saturate it. The amyl alcohol layer got a violet colour and this, on addition of a drop of alcoholic $FeCl_3$, turned blue.

(2) A small portion was shaken with the cyanidin reagent. The upper layer got a tinge of rose colour.

(3) Oxidation test:—A 10 per cent alkaline (NaOH) solution was added to it in presence of air, immediately followed by con. HCl and amyl alcohol, the amyl alcohol layer developed a rose colour.

(4) Circular paper chromatography (horizontal) was carried out using phenol-water (lower layer) as the irrigating solvent at 28°C. A prominent red ring was observed having Rg=0.70. A sample of cyanidin chloride prepared from rose petals gave Rg=0.70 under similar conditions.

The ethanolic hydrochloric acid solution of the anthocyanidin chlorides prepared from A and C showed an absorption maximum at 547 mμ. Thi value nearly corresponds to the standard value of absorption maximum of cyanidin chloride (545 mμ). All the above facts indicated that the anthocyanidin chloride studied was cyanidin chloride.

The solubility and colour reactions showed that the compound B might also be a flavonoid, e.g. leucoanthocyanin (glycoside). *Detection of the sugar residue in the glycoside* (Compound B).—

The substance (1g) was refluxed with 7 per cent aqueous H_2So_4 (25 ml) for 45 min. The contents were then cooled and the phlobaphene was removed by filtration. The colouring matter was removed by isoamyl alcohol. The

aqueous portion was neutralised with solid $BaCo_3$ and the solution was filtered and concentrated. A drop of the solution when subjected to circular paper chromatography using phenol saturated with water as the irrigating solvent and aniline hydrogen phthalate as the developer, gave a ring with Rg=0 58, which was in agreement with that given by an authentic sample of glucose under the same conditions.

Compound B on boiling with alcoholic hydrochlorie acid produced only phlobaphene and no anthocyanidin chloride was detected in the amyl

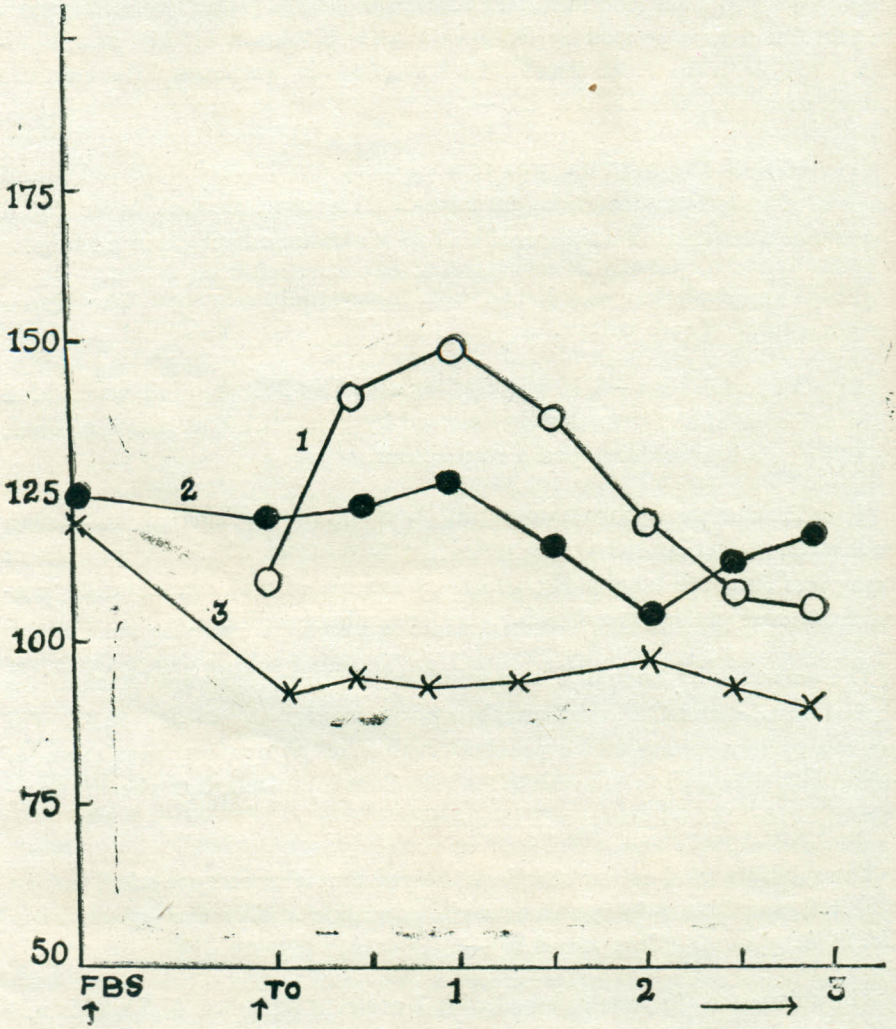

Fig. I. Influence of the drugs on glucose tolerance.
1. Glucose alone. 2. F. bengalensis. 3. Tolbutamide.

TABLE I

Hypoglycemic effects of different Compounds isolated from F. bengalensis Linn, compared with that of tolbutamide

Substance administered orally (0.25 g/Kg).	Blood sugar (mg/100 ml) Mean values for six rabbits in each group					Maximum reduction % in blood sugar	Maximum hypoglycemic effect as % of tolbutamide (Mean of a & b)	Statistical analysis.
	F.B.S.	1 hr	2 hr	3	4 hr			
Tolbutamide	(a) 112.9±5.4	100.1±4.9	92.4±5.1	81.1±5.5	65.2±4.2	42.2	.....	t=2.642
	(b) 118.2±6.1	105.2±5.2	85.1±5.5	75.1±6.2	63.2±5.1	46.5	.....	p>0.02
Compound A	(a) 115.2±5.9	110.36.8	101.2±5.8	98.1±5.4	99.2±5.7	14.9	36.4	t=2.845
	(b) 120.1±6.1	113.4±7.1	105.3±6.6	99.2±642	103.5±6.1	17.4		p>0.02
Compound B	(a) 122.2±7.2	110.1±6.8	100.0±5.1	95.2±5.9	96.3±6.2	22.0	48.5	t=0.808
	(b) 114.1±6.8	100.5±5.3	92.3±5.5	90.1±5.7	95.2±6.2	21.1		p>0.5
Compound C	(a) 110.1±6.4	105.1±7.2	100.2±6.8	98.2±5.9	104.5±5.7	10.8	23.63	t=0.9053
	(b) 120.5±6.4	115.2±6.5	113.5±5.9	115.2±6.2	108.2±6.5	10.2		p<0.4

alcohol soluble fraction. The absence of gallic or ellagic acid in the hydrolysed product as shown by the chromatographic analysis using the organic phase of n-butanol : acetic acid : water mixture as irrigating solvent, and $FeCl_3$ as developer, indicated that the glucoside was either some leucoanthocyanin which resisted conversion into the corresponding anthocyanidin chloride or a compound of some condensed tannin.

The hypoglycemic effects of these three flavonoids A, B and C were then compared with tolbutamide by the usual procedure of biological assay as followed previously (Brahmachari and Augusti, 1961). Results given in Table I indicate that Compound B is the most effective of these three compounds. Fig. I indicates the effect of compound B in controlling the experimentally induced hyperglycemia by glucose administration in normal fasting rabbits studied according to the usual procedure (Brahmachari and Augusti 1962).

DISCUSSIONS

The physiological properties of the flavonoids and related compounds have not yet been fully established. But various properties viz. bacteriostatic action, diuretic action, cardiac stimulation, vasoconstriction etc. (Seshadri 1951), have been reported from time to time. It will be interesting, therefore, to add the hypoglycemic effect noted for these principles to the list of known physiological properties of such compounds.

The authors wish to gratefully acknowledge the help and guidance received from Prof. T.R. Seshadri F.R.S. and Dr. V.V.S. Murti of the University of Delhi. Financial assistance from M/s Charles E. Frosset, Canada, is thankfully acknowledged.

REFERENCES

Agri. Ledger. Calcutta (1904). No. **4**, 26.

Bate and Smith E.C. (1954). *Biochem J.*, **58**, 122.

Brahmachari, H.D. and Augusti, K.T. (1961). *J. Pharm. Pharmacol.*, **13**, 128 and 381.

Brahmachari, H.D. and Augusti, K.T. (1962). *J. Pharm. Pharmacol.*, **14**, 254 and 617.

Deshmukh. V.K., Shrotri, D.S. and Aiman. R. (1960). *Ind. J. Physiol. Pharmacol.*, **4**, 182.

Rosenheim, O. (1920). *Biochem. J.*, **14**, 178.

Robinson, G.M. and Robinson, R. (1933). *Biochen. J.*, **27**, 206.

Sharma, J.N. and Seshadri, T.R. (1955). *J. Sc. Ind. Res.*, **14B**, 211.

Seshadri, T.R. (1951). *Ann. Reviews Biochem.*, **20**. 488.