Phytochemical and Hypoglycemic Investigation of *Casearia esculenta*

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In the course of a systematic investigation of *Casearia esculenta*, the following components were isolated: a sterol, m.p. 132–134°; a substance resembling gutta-percha, m.p. 59–60°; a flavonoid compound, m.p. 255–258°; a colorless crystalline compound, neutral and water-soluble, m.p. 184°; and two resins. A reducing sugar present in the drug was identified by paper chromatography as arabinose. The hypoglycemic activity of the different fractions, both isolated and in alcoholic and aqueous extracts, has been studied.

THE ROOTS OF *Casearia esculenta* Roxb. (N. O. *Samydaceae*) are used as an antidiabetic drug in southern and southwestern India, particularly in Maharastra, by physicians who practice the Indian system of medicine known as Ayurvedha. The drug is considered to be a specific remedy for diabetes (1), and is widely used throughout Decan (2). A search of available literature (1, 3, 4) and abstracts revealed little or no scientific information concerning the evaluation of the medicinal properties of the plant. The present investigation was undertaken primarily on the basis of a preliminary report (5) which indicated the presence of hypoglycemic activity in a water-soluble crystalline body *S*, m.p. 182°, and also in two resin fractions, one of them water soluble but acid insoluble. From the available experimental data it is now evident that a water-soluble crystalline body, m.p. 184°, isolated from the roots of the plant, is responsible for the hypoglycemic activity.

EXPERIMENTAL

Source and Preparation of Material—Drug plant roots used in these investigations were received through the courtesy of Sri K. G. Gune, Ayurvedhashram Pharmacy, Ahmednagar, and authenticated by Sri Balwant Singh, Ayurvedic Garden, Banaras Hindu University, by comparison with the samples kept at the herbarium of the Ayurvedic Division, College of Medical Sciences, Banaras Hindu University. The roots were reduced to moderately fine powder.

Percentage of Extracts and Ash Determination—Suitable samples of the powdered drug were extracted successively with a series of selective solvents in a Soxhlet apparatus according to a modified Rosenthaler procedure (6). The solvents utilized and the per cent of extracts obtained with each solvent were: petroleum ether (b.p. 40°–60°), 2.55%; ether, 0.73%; benzene, 0.75%; chloroform, 0.15%; acetone, 2.66%; and alcohol, 5.34%. The determinations for ash content as recommended by the B.P. (7) afforded the following results: total ash, 3.25%; acid-insoluble ash, 0.243%; and water-soluble ash, 0.58%.

Extraction of the Drug with Benzene and Alcohol and Separation of Different Fractions (Scheme I)—Ten kilograms of the drug was completely extracted with benzene, and the extract was concentrated and ultimately dried. The residue obtained was labeled as fraction A.

The residue left after the extraction with benzene was further extracted with alcohol by percolation. The total alcoholic extract was concentrated under reduced pressure to a small volume and set aside. Some solid matter deposited from the concentrated extract and was separated from time to time. This process was continued until there was no further deposition on standing. The mixed solid was washed with a little cold alcohol and then with dry ether. It was labeled as fraction B. The ethereal washings gave positive tests for sterols and was labeled as fraction C. It was examined along with fraction A.

The alcoholic extract left after the removal of fraction B was poured in a thin stream, with constant stirring, into a large volume of water. The precipitated mass was separated, dried, and labeled as the resin. The supernatant liquid after acidification with dilute hydrochloric acid gave another precipitate which was collected and dried. This fraction was labeled as the acid-insoluble resin.

The filtrate gave positive tests for reducing sugars. A portion, labeled as fraction F, was used for the identification of the sugar.

Isolation of Sterol—*From Fraction A*—A suitable amount of fraction A was saponified with alcoholic potash and the unsaponifiable portion, after removal of alcohol, was taken up in ether. No crystalline matter separated from the ethereal extract even on prolonged storage at low temperature (10°). On addition of acetone to the ethereal extract, one yellowish body was precipitated. The precipitate gave faint tests for sterols. It was purified by repeatedly dissolving in ether and reprecipitating with the addition of acetone. After purification, this solid failed to respond to any of the tests for sterols. This was labeled as fraction K. After the removal of fraction K, only one sterol, m.p. 132–134°, could be isolated from the mother liquor.

From Fraction C—A sterol, m.p. 132–134°, separated during attempts at crystallization from

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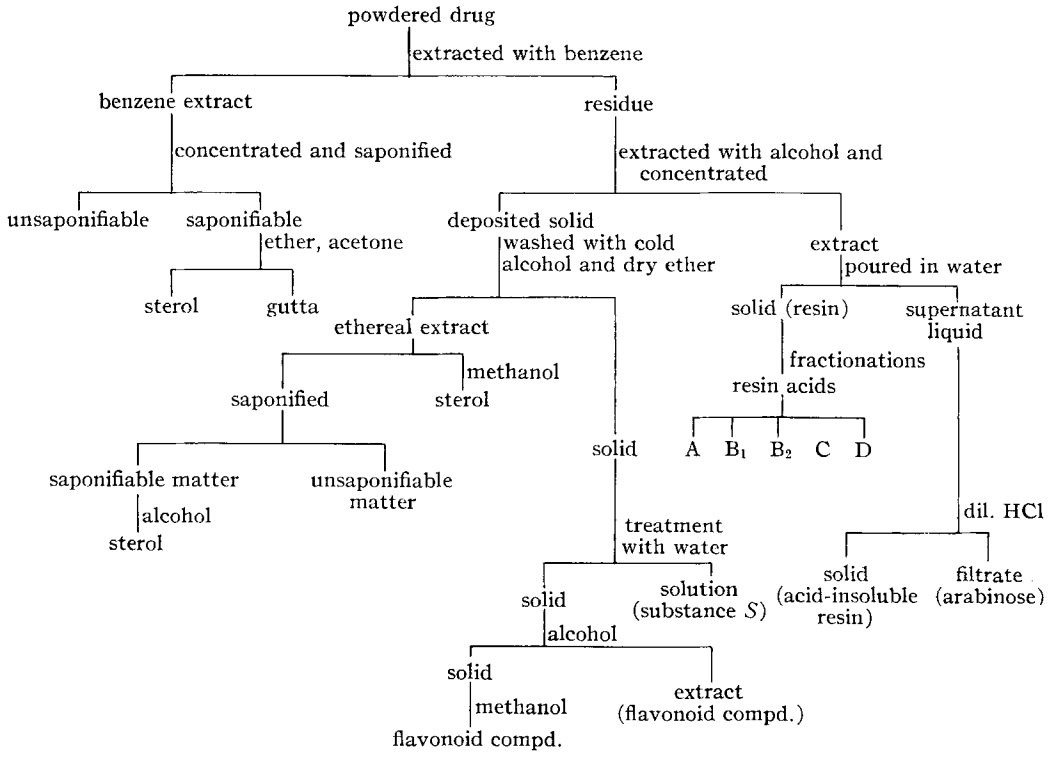

Scheme I

methanol and also by the process of saponification and taking up in ether and ultimate crystallization from ethanol. All the sterol fractions were found to be the same since there was no depression when mixed melting points were taken.

The following derivatives of the sterol were prepared and their melting points noted: digitonide, m.p. 202–203°; acetyl, m.p. 113–114°; and benzoyl, m.p. 113–116°.

Anal.—Calcd. for $C_{24}H_{38}O_2$: C, 80.44; H, 10.61. Found: C, 80.71; H, 10.47.

Molecular weight (Rast) using camphor as solvent.

Gutta-Like Substance—Fraction K, found during the isolation of sterol, was found to melt at 59°–60°. Elemental analysis (8) indicated the absence of nitrogen, sulfur, phosphorus, and halogens. It was classified [as per Tracey (9)] as gutta from its physical behavior and chemical tests. Since this substance did not show any hypoglycemic activity, it was not studied further.

Isolation of Substance S—Fraction B was treated with hot water. The suspension was filtered and the residue was repeatedly treated with warm water until a small volume of the filtrate did not leave anything on evaporation. The residue which did not go into solution was marked fraction P and set aside. A flavonic compound, isolated from fraction P, is described later. The mixed filtrate was concentrated to a small volume and kept for crystallization. A number of crops of crystals, m.p. 178–179°, were isolated. The crops were recrystallized separately from water and their melting points were found to lie between 182–184°. The mixed melting points of different crops did not show any depression. All the crops were, therefore, mixed together and dissolved in water and allowed to crystallize slowly. The crystals, as they appeared, were collected separately in several lots. The melting points of first and last fraction were taken and found to be 184°. When tested for hypoglycemic activity, this product was found to be active both on normal rats (Table VI) and rabbits (Table VII). The chemical constitution and antidiabetic activity of this compound, named substance *S*, (C, 39.6; H, 6.82) will be reported in a separate communication.

Isolation of Flavonoid Compound—Fraction P was found to have a silky appearance in water. It decolorized acid permanganate solution and gave positive tests for phenols with a solution of ferric chloride. Five grams of fraction P was refluxed several times with ethanol which took a small portion of it in solution. From the alcohol-soluble portion a granular body crystallized, m.p. 246–251° dec. This product turned green in a solution of ferric chloride. Light yellow needle-shaped crystals, m.p. 254–258°, with repeated crystallization were obtained from the granular body. This was labled fraction P/alcohol.

The mass left after refluxing with alcohol was refluxed several times with methanol. From the mixed methanolic extract a yellow crystalline product separated on cooling. This was separated and repeatedly crystallized from hot methanol until there was no rise in the melting point, 255–257°. This product was also found to turn green

TABLE I—CONCENTRATION OF BLOOD SUGAR AFTER A SINGLE ORAL DOSE OF ALCOHOLIC EXTRACT (1:10)[a]

Rat	Initial Blood Sugar, mg./100 ml.	Blood Sugar, mg./100 ml. After 2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
			Drug			
1	110.8	99.20	91.82	97.01	100.6	103.5
2	113.5	100.3	94.14	97.87	101.5	104.6
3	124.1	107.5	101.3	105.9	109.8	114.2
Mean % variation		−11.82	−17.52	−13.63	−10.42	−7.47
			Control			
1	118.4	117.1	116.6	114.7	114.2	113.1
2	115.9	114.8	114.4	112.6	112.1	111.6
3	112.2	111.1	110.7	108.8	108.2	107.2
Mean % variation		−1.00	−1.37	−3.00	−3.46	−4.21

[a] Dose, 25 ml./Kg.

TABLE II—CONCENTRATION OF BLOOD SUGAR AFTER A SINGLE ORAL DOSE OF AQUEOUS EXTRACT (1:10)[a]

Rat	Initial Blood Sugar, mg./100 ml.	Blood Sugar, mg./100 ml. After 2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
			Drug			
1	125.4	106.9	100.8	102.6	109.7	115.7
2	119.4	102.7	98.86	100.0	104.4	111.5
3	102.8	89.14	84.91	85.86	88.94	96.22
Mean % variation		−13.98	−18.08	−17.02	−12.86	−6.91
			Control			
1	116.3	115.6	114.5	113.1	112.8	111.9
2	114.6	113.3	111.7	108.8	109.6	108.5
3	105.7	104.8	103.9	102.8	102.6	100.2
Mean % variation		−0.86	−1.92	−3.52	−3.43	−4.67

[a] Dose, 25 ml./Kg.

TABLE III—CONCENTRATION OF BLOOD SUGAR AFTER A SINGLE ORAL DOSE OF CRUDE RESIN[a]

Rat	Initial Blood Sugar, mg./100 ml.	Blood Sugar, mg./100 ml. After 2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
			Drug			
1	105.7	98.80	93.60	93.10	95.40	99.60
2	113.7	103.3	97.45	98.23	103.5	105.5
3	110.2	101.5	94.63	97.82	99.70	101.6
Mean % variation		−7.73	−12.75	−11.63	−9.23	−6.81
			Control			
1	110.2	108.7	107.9	107.1	105.8	104.3
2	118.6	116.9	115.3	114.2	112.4	112.1
3	121.3	120.2	118.5	117.7	115.9	115.3
Mean % variation		−1.23	−2.39	−3.16	−4.55	−5.26

[a] Dose, 2 Gm./Kg.

TABLE IV—CONCENTRATION OF BLOOD SUGAR AFTER A SINGLE ORAL DOSE OF CRUDE ACID-INSOLUBLE RESIN[a]

Rat	Initial Blood Sugar, mg./100 ml.	Blood Sugar, mg./100 ml. After 2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
			Drug			
1	126.7	119.2	113.8	113.0	117.2	120.1
2	119.2	113.5	107.1	105.3	111.1	113.8
3	100.6	95.32	91.35	91.60	94.29	97.25
Mean % variation		−5.31	−9.50	−10.31	−6.85	−4.41
			Control			
1	115.6	114.8	114.6	112.3	110.7	109.4
2	123.3	122.5	121.8	119.8	118.6	118.1
3	121.7	120.4	120.1	118.6	117.8	117.3
Mean % variation		−0.80	−1.06	−2.71	−3.74	−4.39

[a] Dose, 2 Gm./Kg.

TABLE V—CONCENTRATION OF BLOOD SUGAR AFTER A SINGLE ORAL DOSE OF ETHER-INSOLUBLE PORTION OF RESIN[a]

Rat	Initial Blood Sugar, mg./100 ml.	Blood Sugar, mg./100 ml. After 2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
			Drug			
1	112.8	103.0	97.73	96.65	100.9	105.3
2	108.9	101.6	96.65	96.42	99.0	102.3
3	118.5	107.2	103.0	106.6	106.2	111.0
Mean % variation		−8.24	−12.56	−11.34	−9.84	−6.62
			Control			
1	119.6	118.4	117.8	116.9	116.6	114.8
2	128.3	126.9	126.1	124.3	122.8	122.5
3	107.5	106.1	105.4	104.7	103.9	103.1
Mean % variation		−1.13	−1.87	−2.65	−3.37	−4.20

[a] Dose, 2 Gm./Kg.

TABLE VI—CONCENTRATION OF BLOOD SUGAR AFTER A SINGLE ORAL DOSE OF SUBSTANCE *S* IN RATS[a]

Rat	Initial Blood Sugar, mg./100 ml.	Blood Sugar, mg./100 ml. After 2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
			Drug			
1	123.9	106.2	95.66	99.27	104.1	111.4
2	105.3	90.48	82.93	84.85	87.54	93.63
3	119.8	104.2	93.28	98.43	101.8	108.9
Mean % variation		−13.79	−12.23	−19.06	−16.0	−10.07
			Control			
1	117.1	115.5	114.8	112.6	111.9	110.3
2	113.6	112.0	111.0	108.7	107.7	106.6
3	114.8	113.8	112.5	111.0	110.1	109.9
Mean % variation		−1.21	−2.08	−3.88	4.57	−5.55

[a] Dose, 0.5 Gm./Kg.

in a solution of ferric chloride and labeled as fraction P/methanol.

Both fraction P/alcohol and P/methanol exhibited the following colors: (*a*) ferric chloride solution, green; (*b*) magnesium and HCl, orange red; (*c*) boric acid and HCl, yellow; (*d*) sodium hydroxide solution, greenish yellow; (*e*) neutral lead acetate, yellow. As the above color reactions were characteristics of flavanoids (10), the method of Gupta and Seshadri (11) for the isolation of flavanoid compounds was adopted and one crystalline yellow product, m.p. 255–258°, was isolated. This fraction was also found to respond to all the color tests mentioned above. All the three fractions were then subjected to paper chromatography, and the chromatograms were developed with various reagents (10). The same color and R_f values were obtained with all the fractions. Mixed melting points of the different fractions did not show any depression. This indicated that they were identical. Microcombustion analysis gave the following results: C, 54.02; H, 3.88. These fractions were found to be inactive when tested for hypoglycemic activity and were not further investigated.

Studies with Resin Fractions—Both the resin fractions reported above were found to lower the blood sugar level of normal albino rats (Tables III and IV). But on purification this hypoglycemic property was found to have disappeared completely from both the fractions. However, the portion that did not go into solution in ether during fractionation of the resin was found to have hypoglycemic activity (Table V). This indicated that the hypoglycemic activity might be due to adsorption of substance *S* in the crude resin fractions. On fractionation by a chemical method (12), the following resin acids were isolated from the resin: (*a*) resin acid A, m.p. 147–164° (with frothing); (*b*) resin acid B_1, m.p. 85–91° (with frothing); (*c*) resin acid B_2, m.p. 81–132° (with frothing); (*d*) resin acid C, m.p. 144–150° (with frothing); (*e*) resin acid D, m.p. 130–144° (with frothing).

Identification of Reducing Sugar—The reducing sugar present in the drug was identified as arabinose by paper chromatography (13). This was done by making separate chromatograms of a pure sample of arabinose, fraction F, and a mixture of fraction F and added arabinose. After development, each showed only one spot at the same R_f value.

Hypoglycemic Studies—Healthy albino rats of the same breed and of nearly the same weight range were selected for carrying out the experiments. They were divided into two groups: one for control and the other for the drug. Food was withdrawn 18 hr. before starting the experiments, and no food was given during the experiment. The drug was given *per os*. Blood samples were taken from the tails of the animals and blood sugar was determined by the method of Folin and Wu (14). A color comparison was made in a Lumetron photoelectric colorimeter (Lumetron model 402EF). Of all the fractions tested, only the alcoholic and aqueous extracts, two crude resin fractions, and substance *S* were found to be active, and the results

TABLE VII—CONCENTRATION OF BLOOD SUGAR AFTER A SINGLE ORAL DOSE OF SUBSTANCE *S* IN RABBITS[a]

Rabbit	Initial Blood Sugar, mg./100 ml.	Blood Sugar, mg./100 ml. After 2 hr.	4 hr.	6 hr.	8 hr.	10 hr.
			Drug			
1	120.4	102.9	97.80	101.6	107.3	110.6
2	118.3	103.5	98.30	103.1	108.3	108.6
3	112.4	103.8	96.60	105.2	108.1	112.2
Mean % variation		−14.53	−19.12	−14.63	−10.68	−8.09
			Control			
1	113.8	112.7	112.3	111.5	110.4	109.9
2	116.5	115.0	114.4	113.3	112.8	112.4
3	121.1	119.0	118.3	117.9	117.3	116.3
Mean % variation		−1.32	−1.81	−2.44	−3.10	−3.64

[a] Dose, 0.5 Gm./Kg.

obtained with these fractions are given in the Tables I–VII.

From the observations recorded in the tables, it is quite evident that the hypoglycemic activity of the drug is due to substance *S* alone. Experiments to prove the antidiabetic activity of substance *S* are presently in progress.

SUMMARY

A number of fractions were isolated by a chemical method from the drug, *C. esculenta*. All of the isolated fractions were tested biologically for their hypoglycemic activity and only a few fractions, *viz.*, the two crude resin fractions and a water-soluble crystalline body *S*, showed positive results. On purification, both the resin fractions proved to be inactive, and this indicated that the activity might be due to adsorption of substance *S* in the crude resins. This was further confirmed by the fact that when the crude resin was extracted by ether the portion that did not go into solution in ether gave a positive response.

The chemical constitution and the antidiabetic activity of substance *S* will be presented in another paper.

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