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Exploratory Studies on the Constituents of *Scoparia dulcis* (Linn)

By WALTER M. BUDDE * AND R. L. SHRINER

INTRODUCTION

In 1942 M. C. Nath of the University of Dacca in India reported that an Indian drug called amellin was effective orally in treating diabetes (1). From 1942-1947, seven additional papers have been published by Nath and co-workers (2) which describe the physiological effects of amellin and also clinical results on 15 patients. It is stated that oral doses of 15 to 20 mg. per day along with calcium salts over a period of several weeks to three months cause return of blood sugar to normal levels and disappearance of glycosuria and ketonuria.

The only information about amellin given, was that it was obtained from a plant *Scoparia dulcis* (Linn) and that it was not an alkaloid. No details of extraction, isolation or composition were published. The object of the present work was to explore the possibility of obtaining physiologically active extracts of this plant.

EXTRACTION AND SEPARATION OF FRACTIONS

The plant, *Scoparia dulcis* (Linn) was obtained from India through the courtesy of a representative of the Eli Lilly & Co. Its botanical identity was verified by a botanist in India at the time of collection. About 14 lb. of the entire dried plant was ground in a Wiley mill and the powder subjected to a series of extractions which may be summarized as follows:—

Fraction A. In order to remove oils, fats and waxes, five successive extractions of the powder with petroleum ether were carried out. Distillation of this petroleum ether extract left a brown waxy solid. This amounted to about 1% of the weight of the plant and was a mixture of fats, waxes and sterols which were not separated but designated as Fraction A.

Fraction B. The residue left after removal of Fraction A, was successively extracted with boiling 95% ethanol. Distillation of this solvent left a dark green semi-solid mass. It was triturated with petroleum ether which removed additional fats and waxes and left a residue of a dark green sticky solid:— Fraction B. It had no distinct melting point and gave weak tests for nitrogen and chlorine but no sulfur or phosphorus. Many attempts were made to

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obtain crystalline material from this fraction B, which amounted to 2.5% of the plant, but no pure compounds could be obtained. Fraction B was soluble in alkalis and reprecipitated by acids. After attempted hydrolysis with dilute acids, no sugars could be detected.

Fraction C. Continuous extraction of Fraction B in a Soxhlet apparatus with chloroform for 20 hours removed additional amounts of wax and also traces of nitrogen compounds. The insoluble material designated as Fraction C was a brown solid which had no definite melting point. For purification it was acetylated with acetic anhydride and pyridine. This reaction mixture was poured into ice and hydrochloric acid, the precipitate removed by filtration and purified by solution in acetone, treatment with Norite, filtration, and precipitation with water. This gave a light brown powder which melted indistinctly around 125°. It contained no nitrogen or halogen and was a compound of carbon, hydrogen and oxygen.

Analyses: C, 58.75%; H, 5.66%

Percent Acetyl, 25.5%; Mol. wt. 568 ± 10

These correspond reasonably well with the molecular formula $C_{28}H_{32}O_{13}$ which may be expanded to $C_{22}H_{23}O_7(OCOCH_3)_3$ or $C_{20}H_{20}O_5(OCOCH_3)_4$ since the percent acetyl falls between 3 and 4 acetoxy groups. The original Fraction C would be $C_{22}H_{26}O_{10}$.

This Fraction C and its acetyl derivative differed markedly from the naturally occurring glucoside, phloridizin and its acetate. Further experiments designed to purify Fraction C did not yield any definite compounds.

Other Fractions. After removal of Fraction C, the residue was extracted with 49% ethanol — 49% water — 2% hydrochloric acid solution. This yielded a dark black extract (Fraction D) which was subjected to a series of extractions from acid and alkaline media followed by acetylation. However, no pure homogeneous compounds could be obtained.

PHARMACOLOGICAL TESTS

Through the courtesy of the Pharmacology Division of the Eli Lilly Company, the activity of these fractions was studied. The results may be summarized as follows: —

1. Hot water extracts of the whole plant were administered by stomach tube to 6 fasting rabbits. Three showed a slight increase in blood sugar and three showed a slight decrease. The variations were within those occurring in different animals.

2. Fraction A, caused a slight fall in blood sugar of two rabbits when given orally in a capsule but the drop occurred about 5 to 6 hours after administration and was within variations normally noted in fasting rabbits.
3. Fraction B. Administration of 1 g. of this product caused drop of blood sugar in fasting rabbits from 100-105 mg./100 cc. to 78-80 mg./100 cc. at the end of 7 hours.
4. Fraction C caused an erratic drop in blood sugar at various times.
5. Acetylated Fraction C. This derivative caused a consistent drop of 30 to 35 mg. in blood sugar of two rabbits over a 7 hour period. However, the change in sugar content was not proportional to the amount given, and no signs of typical shock due to this lowering of blood sugar were noted. The behavior was not like that of insulin.
6. Fraction C was further purified by treatment with hydrochloric acid, the residue dissolved in alkali, filtered and reprecipitated by acid. The tests on this fraction on fasting rabbits were similar to Fraction C. This material was also given to three rats rendered diabetic by administration of alloxan. The average daily food consumption, urine output and sugar excreted in the urine were found to agree with the controls within experimental error.

SUMMARY

The results indicate that none of the fractions obtained possessed a true insulin like effect. The changes noted were all within variations in blood sugar normally found.

In view of the reports by Nath, the failure to find definite hypoglycemic compounds may be due to: (a) the Indian drug amellin was obtained from some other variety of *Scoparia dulcis* (Linn); (b) the amellin may be present in the fresh growing plant only, whereas the above extracts were on dried material; (c) the improvement of the patients observed by Nath was due to other factors such as improved diet.

Addendum. After the above work was completed an abstract of an Indian patent issued to M. C. Nath (3) appeared which stated that the fresh green plant was extracted with water or water and alcohol mixtures, the extract purified and the residue obtained by evaporation used as the active principle.

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3. Nath, Indian Pat. 36, 407 dated June 9, 1948.

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