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SHORT COMMUNICATION

# THE SUGARS IN THE EXTRAFLORAL NECTAR OF *ANDROPOGON GAYANUS* VAR. *BISQUAMULATUS**

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**Abstract**—The sugars occurring in the extrafloral nectar of *Andropogon gayanus* var. *bisquamulatus* were examined by paper and gas chromatography. They were found to be principally sucrose, glucose and fructose with small amounts of maltose, raffinose, arabinose, xylose and three unidentified sugars.

## INTRODUCTION

NECTAR IS secreted by a wide range of flowering plants and at least one fern. The secretory glands are found predominantly in the flowers, when they are termed floral nectaries, or less commonly on other parts of the plant such as the petioles, when they are termed extrafloral nectaries. Nectar secretion is less common in the monocotyledons than in the dicotyledons and in the Gramineae it is rare. Thus floral nectaries have never been reported in grasses and extrafloral nectaries only in the genera *Eragrostis* and *Andropogon*.[1] None of these nectars appears to have been analysed. For these reasons this investigation was made on the extrafloral nectar found to be secreted by *Andropogon gayanus* var. *bisquamulatus* (Hochst.) Hack. (Gramineae).

This plant is a large, perennial, African savannah grass[2,3] and has been grown at Chelsea in a heated, illuminated greenhouse for over 5 yr. Under these conditions it grows very vigorously without flowering. The tillers produced become increasingly large and luxuriant until, in year 3 after sowing, they may reach 15 mm dia. 100 cm height. It is at this stage of growth that they begin to produce extrafloral nectar during the summer and autumn. At the junction of the leaf sheath with the pseudopetiole the sheath margin bears a pair of darkly pigmented shoulders. Single drops of nectar, up to 3 mm dia., appear at each pigmented area and rows of much smaller drops may also appear along the adjacent pseudopetiole ridges.

There seems to be no fundamental difference between floral and extrafloral nectars.[4] Percival[5] classified all nectars on the basis of their major sugars as: (*a*) dominant sucrose nectars when sucrose is clearly the major constituent; (*b*) balanced nectars when sucrose, fructose and glucose are present in nearly equal quantities; and (*c*) dominant fructose–glucose nectars when these two sugars are clearly the major constituents.

* Part V in the series "Studies on *Andropogon gayanus*".

[1] G. E. MATTEI and C. TROPEA, *Boll, R. Orto Bot. Giard. Col., Palermo* **7**, 113 (1908).
[2] B. N. BOWDEN, *J. Ecol.* **52**, 255 (1964).
[3] B. N. BOWDEN, *J. Linn. Soc.* **58**, 509 (1964).
[4] G. R. WYKES, *New Phytologist* **51**, 210 (1952).
[5] M. S. PERCIVAL, *New Phytologist* **60**, 255 (1961).

Nectars may also contain small quantities of other sugars such as raffinose, mellibiose, and maltose; some of them as yet unidentified. Mucilage, protein, enzymes, organic acids and water-soluble vitamins may also be present.

Most analytical work on nectar sugars has been carried out on floral nectars using paper chromatography and has been confined to qualitative work with subjective estimates of the sugar concentrations. GLC of the trimethylsilyl derivatives of sugars[6] now offers a far more powerful technique for routine qualitative and quantitative analysis of nectar sugars and has already been used on the extrafloral nectars of orchids.[7]

## RESULTS AND DISCUSSION

Drops of nectar were detached from the leaves by a small spatula, dried in a desiccator and examined by paper chromatography. A solution of 10 mg nectar/ml 80% ethanol was applied to the paper in a range of loadings and the sugars separated in ethyl acetate–acetic acid–formic acid–water. Three strong spots were detected which co-chromatographed with fructose, glucose and sucrose and three faint spots, two of which co-chromatographed with maltose and raffinose.

A more detailed qualitative and quantitative analysis of the nectar sugars was made using gas chromatography. 5-mg samples of the nectar were dissolved in pyridine and converted to the trimethylsilyl derivatives,[6] the pyridine then being evaporated off. The silanized sugars were then redissolved in $CS_2$ for injection into the Perkin–Elmer F11 gas chromatograph. The 2 m E301 column was programmed to run isothermally at 140° to separate the monosaccharides then after 13 min to begin a temp. gradient of 10°/min up to 290°, to separate higher saccharides.

Eleven peaks, *A–K* (Table 1), could be distinguished on the gas chromatogram and probable identifications were made by comparing their relative retention times with those of standard silanized sugars obtained under the same conditions. The largest peaks were *D*, *E*, *F*,

TABLE 1. GAS CHROMATOGRAPHY OF SUGARS IN THE EXTRAFLORAL NECTAR OF *Andropogon gayanus* VAR. *bisquamulatus*

| GLC peak | Sugar | Relative quantities of sugars | | | |
|---|---|---|---|---|---|
| | | Fructose = 100 | | Sucrose = 100 | |
| *A* | Arabinose | 4·1 | | 2·4 | |
| *B* | Xylose | 4·5 | | 2·6 | |
| *C* | Unidentified | 8·5 | | 4·9 | |
| *D* | Fructose | 100·0 | | 57·9 | |
| *E* | α-Glucose | 47·5 | 108·0 | 27·5 | 62·5 |
| *F* | β-Glucose | 60·5 | | 35·0 | |
| *G* | Unidentified | 23·9 | | 13·8 | |
| *H* | Unidentified | 30·3 | | 17·5 | |
| *I* | Sucrose | 172·6 | | 100·0 | |
| *J* | Maltose | 3·6 | | 2·1 | |
| *K* | Raffinose | 2·5 | | 1·4 | |

[6] C. C. SWEELEY, R. BENTLEY, M. MAKITA and W. W. WELLS, *J. Am. Chem. Soc.* **85**, 2497 (1963).
[7] S. I. BASKIN and C. A. BLISS, *Phytochem.* **8**, 1139 (1969).

and *I* which, on this basis, appear to be respectively fructose, α-glucose, β-glucose and sucrose. Peaks *G*, *H*, and *J* are most probably disaccharides. *J* co-chromatographs with maltose which has been reported from other nectars;[5,6] *G* and *H* could not be identified but neither is mellibiose. Peak *K* co-chromatographs with raffinose. Peaks *A* and *B* co-chromatographed with arabinose and xylose but *C* could not be identified. The evidence from the paper chromatograms agrees with the identifications of fructose, glucose, sucrose, maltose and raffinose.

The proportions of the sugars present in the nectar were determined relative to fructose by the formula

$$\frac{\text{peak area of sugar}}{\text{peak area of fructose}} \times 100 = \% \text{ fructose.}$$

Fructose was chosen as the datum as it is a major component without detectable anomalous peaks and its area could be measured without extrapolation when using sufficient quantities of silanized nectar to render the smaller peaks visible on the chromatogram. Under these conditions the sucrose peak had to be extrapolated for measurement with consequent loss of accuracy. As sucrose is often used as the datum the mean fructose based figures were also recalculated to sucrose = 100 to facilitate direct comparisons.

*A. gayanus* var. *bisquamulatus* nectar contains about 70% more sucrose than glucose or fructose. On Percival's[5] classification it is thus a weakly dominant sucrose nectar; she also appears (p. 242) to be of the opinion that in dicotyledons exposed nectaries generally produce dominant fructose–glucose nectar. This is not the case with *A. gayanus* var. *bisquamulatus* nectar or with the thirty orchid extrafloral nectars examined by Baskin and Bliss[7] whose data show that only ten of the species examined had dominant fructose–glucose nectars. Further, the relative proportions of sucrose to glucose and fructose in nectars may be a function of their age and the presence of sucrose hydrolysing enzymes. Thus the proportion of sucrose in nectars may not be an ideal basis for classification.

## EXPERIMENTAL

### *Collection of the Nectar*

The extrafloral nectar was obtained from 3–5-yr-old plants of *Andropogon gayanus* var. *bisquamulatus* (Hochst.) Hack. growing in 20-cm pots of John Innes No. 2 compost mixture. These were originally propagated from seed collected at Shika, Zaria Province, Northern Nigeria, and grown in a heated greenhouse maintained above a min. temp. of 15° and with natural lighting supplemented for 12-hr day by Phillips 400 W mercury vapour lights.

A small scalpel was used to detach individual globules of nectar from the pigmented areas at the tops of the leaf sheaths. This was scraped on to a watch-glass and dried in a desiccator. The dried nectar was crushed and mixed to provide an homogeneous sample. The nectar was collected throughout the summer and autumn of 1969.

### *Paper Chromatography*

A range of 5–100-μl spots of solution of nectar (10 mg/ml of 80% ethanol) was spotted on to Whatman No. 1 or 3 MM chromatography paper and run for 24 hr in EtOAc–HOAc–$HCO_2H$–$H_2O$ (18:3:1:4). The separated sugars were detected by dripping first in a $AgNO_3$–$Me_2CO$ bath followed, after drying, by dipping in a NaOH–EtOH bath.[8]

### *Gas Chromatography of Trimethylsilyl Sugar Derivatives*

The separation of the silanized sugars was conducted on a Perkin-Elmer F11 gas chromatograph fitted with a 2 m 0·125 in. O.D., 2% E 301 column and a flame ionization detector. The carrier gas was nitrogen, fed at a flow rate of 76 ml/min. The instrument was programmed for an injection port temp. of 265°, an initial

[8] W. E. Trevelyan, D. P. Proctor and J. S. Harrison, *Nature* **166**, 444 (1950).

isothermal period of 13 min at 140° followed by a 10°/min rise to 290°. 1·0 or 2·0-$\mu$l samples of the $CS_2$ solution of silanized sugar were injected.

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