Phytochemistry, 1977, Vol 16, pp. 529–532 Pergamon Press Printed in England.

SOLUBLE CARBOHYDRATES OF DRY AND DEVELOPING SEEDS

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(*Revised received* 24 *September* 1976)

Key Word Index—Leguminosae; other angiosperms; developing and dry seeds; galactosyl-sucrose oligosaccharides; free monosaccharides; maltose.

Abstract—Sucrose was present in seeds of 31 species at all ages and stages of their development. The raffinose family of oligosaccharides is present in most mature and dry seeds; tomato and tobacco seeds contain planteose, whereas sesame seeds contain this sugar and a higher member of the planteose series. Cotton seeds contain raffinose, stachyose, verbascose and an unidentified ketose. Free monosaccharides were not detected in any of the dry seeds; although free glucose and fructose were detected in some immature seeds, these sugars decreased in amount and eventually disappeared during seed maturation. Sucrose, stachyose, raffinose and verbascose accumulated, in developing soybeans, in that sequence. Maltose, a sugar rarely found in plant tissues, is present in immature soybean and honey locust seeds but does not occur in the other seeds examined. It increases to a maximum during development, subsequently decreases in amount during maturation and ripening and eventually disappears completely. The petioles of old leaves and stems of the soybean plant contain maltose, but the petioles of young soybean leaves, empty pods, leaf blades and roots do not.

INTRODUCTION

Sucrose and the raffinose oligosaccharides (raffinose stachyose, verbascose, and sometimes ajugose) have been reported as the soluble reserve carbohydrates of some dry legume seeds [1–4]. Other legume seeds contain only sucrose, raffinose and stachyose [5–7], as has been reported for seeds from *Coffea* spp. [8], *Pinus thunbergii* [9] and *Gossypium herbaceum* [10]. Seeds of *Plantago* spp. [11, 12] *Nicotiana* spp. [13], *Sesamum indicum* [14], *Mentha* spp. [15], *Fraxinus excelsior* [16], and *Theobroma cacao* beans [17] have been shown, on the other hand, to contain the trisaccharide planteose, an isomer of raffinose.

Evidence for the existence of free mono- and disaccharides in dry seeds has also been presented. Thus, Meidell [6] and Pazur *et al.* [7] reported that the dry *Glycine max* seeds contain free glucose, fructose and galactose in addition to the raffinose oligosaccharides. Abrahamsen and Sudia [5] reported that soybean seeds contain small, variable amounts of free glucose, fructose and maltose. Free melibiose was also reported to be present in lucerne (*Medicago sativa*), guar (*Cyamopsis tetragonolobus*) and carob (*Ceratonia siliqua*) seeds by McCleary and Matheson [18].

Only a limited number of studies appear to have been made on the sequence of formation of oligosaccharides or on the changes in monosaccharides during seed development. In young developing cotton and other seeds [10], *Phaseolus lunatus* [19], *P. vulgaris* [20], *Vicia faba* and other legume seeds [21], it was observed that the raffinose family of oligosaccharides do not appear in the tissue until the onset of ripening. In studies with soybean, radioactivity from $^{14}CO_2$ was incorporated into maltose during early development, but did not appear in raffinose and stachyose until the drying phase of seed maturation [22].

Maltose was reported to be present in young soybean seeds [22], ripening *Secale cereale* seeds [23] and the water soluble fraction of *Cassia* spp. [24]. There are, however, few reports on the occurrence of this sugar in other plant organs. Quillet and Bourdon [25] reported that the petioles, stems and roots of soybean plants contain free maltose but Phillips and Smith [26], using GLC techniques, did not find it in any soybean tissues and organs they studied at any age or development stage. Maltose has also been reported to be present in the lower leaves of the *Helianthus annuus* [27] and in *Panax quinquefolium* root [28]. Bailey [29] reported that the sugar occurs free in *Trifolium pratense* leaves (but not in stems and petioles) during the night and disappears during daytime.

Thus, although the identity of carbohydrates of seeds has been studied extensively, whether free monosaccharides only occur in some dry seeds and not in others has not been resolved. Similarly, the sequence of accumulation of sugars in developing seeds and the question as to whether free maltose is present in them or in other plant tissues are unresolved. Hence, the study reported here was designed to investigate both the identity of sugars in dry seeds and their changes during development.

RESULTS

Dry seeds

All of the seeds studied contain sucrose. Table 1 shows the distribution of other sugars in various dry seeds. Free monosaccharides were not detected in any of the dry seeds studied.

Developing seeds

Free glucose and fructose were detected in developing

Table 1. Distribution of oligosaccharides in dry seed or cotyledons of various seeds

Seed	Raffinose	Stachyose	Verbascose	Ajugose
LEGUMINOSAE				
Bambarra groundnut (*Voandzeia subterranea*)	+	+	+	–
Bambarra groundnut*	+	+	+	trace
Peanut (*Arachis hypogaea*)	+	+	+	–
Soybean (*Glycine max*)	+	+	+	–
Mung bean (*Phaseolus aureus*)	+	+	+	trace
Black eye pea (*Vigna sinensis*)	+	+	+	–
Alaska pea (*Pisum sativum*)	+	+	+	trace
Adzuki bean (*Phaseolus angularis*)	+	+	+	–
Alfalfa (*Medicago sativa*)	+	+	+	–
Broad bean (*Vicia faba*)	+	+	+	trace
Parkia roxburghii	+	+	+	–
Pithecellobium dulce	+	+	+	–
Carob (*Certonia siliqua*)	+	+	+	–
Kentucky coffee tree (*Gymnocladus dioicus*)	+	+	+	–
Acacia spp.	+	+	–	–
Honey locust (*Gleditsia triacanthos*)	+	+	–	–
CRUCIFERAE				
Collard (*Brassica oleracea* cv. *acephala*)	+	+	+	–
Radish (*Raphanus sativa*)	+	+	–	–
CUCURBITACEAE				
Cucumber (*Cucumis sativus*)	+	+	+	–
CHENOPODIACEAE				
Spinach (*Spinacia oleracea*)	+	–	–	–
GRAMINEAE				
Sorghum (*Sorghum vulgare*)	+	+	–	–
Oat (*Avena sativa*)	+	+	+	–
EUPHORBIACEAE				
Castor bean (*Ricinus communis*)	–	–	–	–
DIPSACACEAE				
Teasel (*Dipsacus fullonum*)	+	+	–	–
MALVACEAE				
Cotton† (*Gossypium arboreum*)	+	+	+	–
Okra (*Hibiscus esculentum*)	+	+	+	–
Sunset hibiscus (*Hibiscus manihot*)	+	+	+	–
Common rosemallow (*Hibiscus palustris*)	+	+	+	–
SOLANACEAE				
Jimson weed (*Datura stramonium*)	+	–	–	–
Tomato (*Lycopersicon esculentum*)	( )	–	–	–
Tobacco (*Nicotiana tabacum*)	( )	–	–	–
PEDALIACEAE				
Sesame (*Sesamum indicus*)	( )	( )	–	–

+ detected; – not detected. ( ) member of planteose family of oligosaccharides present. * Bambarra groundnut seeds obtained from Kenya. † Contains a ketose that migrates between raffinose and stachyose.

sesame, redbud (*Cercis canadensis*), Kentucky coffee tree (*Gymnocladus dioicus*), wild tobacco (*N. sylvestris*), cotton, castor bean, scarlet runner bean (*Phaseolus multiflorus*), *Xanthium chinense* and honey locust seeds. These sugars, however, were not detected in developing mung bean, sieva bean (*Phaseolus lunatus*), soldier kidney bean (*Phaseolus vulgaris*), table cowpea (*Vigna unguiculata*), catjang (*Vigna cylindrica*), peanut (*Arachis hypogea*) oat, sorghum, Jack bean (*Canavalia ensiformis*) and black locust (*Robinia pseudacacia*) seeds. Developing soybean (*Glycine max*) and honey locust seeds were the only ones to contain maltose.

Figure 1a shows the pattern of change in free glucose, fructose and maltose in the developing honey locust seed; similar changes were seen in the free glucose and fructose content of developing sesame seeds. In Fig. 1B, the change in planteose and the higher member of the planteose family of oligosaccharides in developing sesame seeds is given.

The pattern of accumulation of maltose, sucrose and the raffinose oligosaccharides in soybean seeds during maturation is shown in Fig. 2. Neither free glucose nor fructose was detected in the developing soybean seed at any age studied.

DISCUSSION

Sucrose is the only free sugar which occurred ubiquitously in the plant tissues studied here. In spite of the reports on the presence in dry legume seeds of free monosaccharides [5–7, 18], these sugars were not detected by us. If present, they were below the level of detection by the procedure used (i.e. 1 μg equiv. glucose/g seed). Oligosaccharides of the raffinose and planteose series were the only ones observed in the dry seeds studied (Table 1).

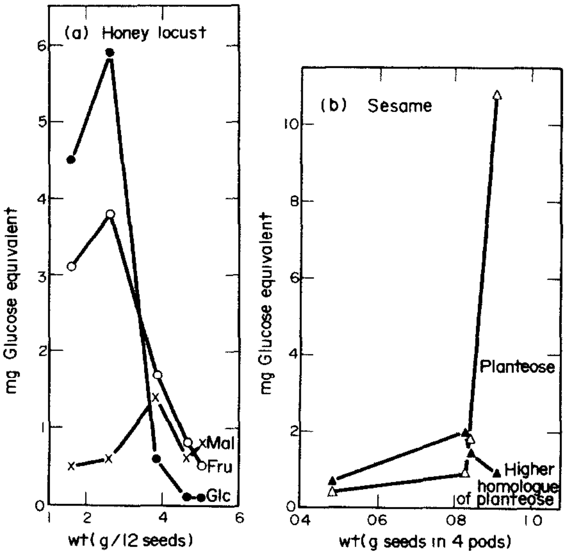

Fig. 1. Patterns of change of sugars in developing honey locust and sesame seeds. Seeds were placed in boiling 80% ethanol, homogenized, extracted and the extracts made to equal volumes. The different sugar components were determined after PC by elution of the areas and reaction with anthrone. a—honey locust; b—sesame. Glc—glucose; Fru—fructose and Mal—maltose.

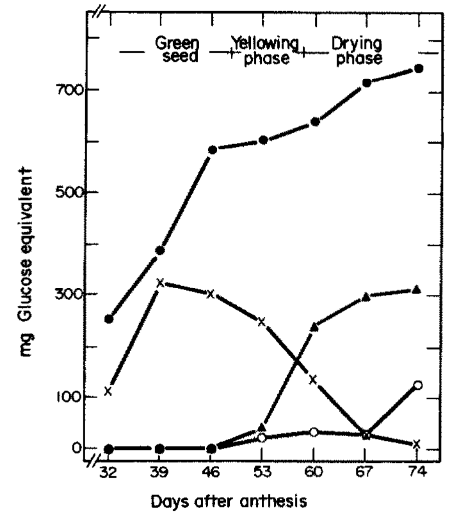

Fig. 2. Patterns of change in oligosaccharides of soybean seeds during maturation. Seeds (40) were taken on the days indicated after anthesis. They were placed in boiling 80% EtOH, homogenized, extracted and the extracts made to equal volumes. Different sugar components for each extract was determined after PC, elution of the areas and reaction with anthrone. ●—● sucrose; ▲—▲ stachyose + verbascose; O—O raffinose and × — × maltose.

It seems likely that the use of water to extract the sugars from soybean seeds by Pazur *et al.* [7] initiated carbohydrase action and this could account for the presence of glucose, fructose and galactose observed in their extracts from dry seeds. Similarly, the procedure used by Abrahamsen and Sudia [5] of soaking the dry seeds in water before extraction could also initiate carbohydrase action which might account for the variable amounts of free glucose, fructose and maltose observed by them. It is also highly probable that the occurrence of free melibiose in lucerne, guar and carob seeds as reported by McCleary and Matheson [18] was an artifact of the treatment of the extract with strong cation exchange resin for too long a period. We have noted that prolonged exposure of extracts of oligosaccharides from dry legume seeds to cation exchange resin may result in the production of glucose, fructose, melibiose and manniotriose.

We have confirmed that glucose and fructose appear in certain developing seeds, increase in amount, subsequently decrease and eventually disappear. This is also true of maltose in both soybean and honey locust seeds. Whether the maltose is transported into the seeds or synthesized therein is not known. Nor is the function of it or that of glucose and fructose in developing seeds known. Perhaps all of them serve as ready reserves for whatever processes are necessary in maturation or for general metabolic purposes. Maltose may act as a primer or may be involved in the formation of a primer for the synthesis of starch.

Our results differ from those of Quillet and Bourdon [25] in one respect: free maltose was not detected in the roots of the soybean plants. We observed further that maltose was not detected in petioles of the terminal (young) leaves while those of older leaves from lower down on the plant contain appreciable amounts of it (about 37%). Thus, it is probable that Phillips and Smith [26] did not detect maltose in their materials due to the young age of the tissue they studied. It is not clear why maltose was not detected in the roots of the soybean plant we studied. The nocturnal pattern of accumulation of maltose reported for red clover [29] was not observed in the soybean tissue investigated by us.

Finally, we have noted that components of the storage oligosaccharides of both the raffinose and plantose series appear to be deposited in the seeds sequentially during maturation, higher members of the series being deposited first.

EXPERIMENTAL

Seeds used in studies on the distribution of sugars during seed development were harvested and separated into various stages of maturity on the basis of size and other visual characteristics. The developing soybean seeds were harvested from plants whose flowers were tagged as they became fully open.

In the extraction of sugars, the various groups of developing seeds were weighed separately, placed in boiling 80% ethanol and homogenized. Extracts of soybean, honey locust and sesame seeds were made to known volumes and aliquots evaporated to dryness under reduced pressure. The resulting residue was redissolved in a known volume of 80% ethanol before being analysed. Dry seeds or cotyledon powder (about 0.5–1 g) of various seeds were also extracted with hot 80% ethanol. Aliquots of the alcohol extracts were chromatographed to separate the different sugar components. Sugars were separated by PC on Whatman No. 4 paper using: *n*-BuOH–Py–H_2O (6:4:3); PrOH–EtOAc–H_2O (7:1:2); and PrOH–EtOAc–H_2O (4:5:1). The dried chromatograms were sprayed with either the diphenylamine–aniline–phosphoric acid reagent to locate all sugars or with the α-naphthol–phosphoric acid reagent to locate ketoses. For the quantitative estimation of sugars, known volumes of the various extracts were applied to paper at three different points on the origin. Chromatograms were developed in the three systems mentioned above. Sugars were located with the diphenylamine reagent on test strips cut from the outer areas of the chromatogram. They were then eluted from their corresponding positions on the unstained center of the chromatogram in a known volume of water. Total carbohydrate content was determined by the anthrone method. Glucose content was determined by the glucose oxidase assay and fructose with the resorcinol reagent.

Acknowledgements — We thank Dr. John L. Lockwood for supplying soybeans and the staff of the Beal-Garfield Botanic Gardens, Michigan State University for supplying some of the developing seeds used in this study. We also thank Drs Suppiah Sinnadurai and John Nabila of the Department of Crop Science and Geography of the University of Ghana, Mr. and Mrs. Nico Ankudey of Bui Game Reserve, Ghana, the Kenya Ministry of Agriculture and the Food and Agriculture Division of the U.S.A.I.D. Missions to Ghana and Kenya for making the bambarra groundnut seeds available for this research. All other seeds were purchased commercially.

REFERENCES

1. Cooper, R. A. and Greenshield, R. N. (1961) *Biochem. J.* **81**, 6P.
2. Nigam, V. N. and Giri, K. V. (1961) *Can. J. Biochem. Physiol.* **39**, 1847.
3. Pridham, J. B. (1958) *Nature* **182**, 1687.
4. Tanusi, S., Kasai, T. and Kawamura, S. (1972) *J. Jap. Soc. Food Nutr.* **25**, 25.
5. Abrahamsen, M. and Sudia, T. W. (1966) *Am. J. Botany* **53**, 108.
6. Meidell, G. E. (1964) *Diss. Abstr.* **24**, 3085.

7. Pazur, J. H., Shadaksharaswamy, M. and Meidell, G. E. (1962) *Arch. Biochem. Biophys.* **99**, 78.
8. Shadaksharaswamy, M. and Ramachandra, G. (1968) *Phytochemistry* **7**, 715.
9. Hattori, S. and Shiroya, T. (1951) *Arch. Biochem. Biophys.* **34**, 121.
10. Shiroya, T. (1963) *Phytochemistry* **2**, 33.
11. French, D., Wild, G. M., Young, B. and James, W. J. (1953) *J. Am. Chem. Soc.* **75**, 709.
12. Herissey, M. H. (1957) *Bull. Soc. Chimie Biol.* **39**, 1553.
13. French, D. (1955) *J. Am. Chem. Soc.* **77**, 1024.
14. Hatanaka, S. (1959) *Arch. Biochem. Biophys.* **82**, 188.
15. French, D., Youngquist, R. W. and Lee, A. (1959) *Arch. Biochem. Biophys.* **85**, 471.
16. Jukes, C. and Lewis, D. H. (1974) *Phytochemistry* **13**, 1519.
17. Cerbulis, J. (1955) *Arch. Biochem. Biophys.* **58**, 406.
18. McCleary, B. V. and Matheson, N. K. (1974) *Phytochemistry* **13**, 1747.
19. Korytnyk, W. and Metzler, E. (1962) *Nature* **195**, 616.
20. Gould, M. F. and Greenshields, R. N. (1964) *Nature* **202**, 108.
21. Bourne, E. J., Walter, M. W. and Pridham, J. B. (1965) *Biochem. J.* **97**, 802.
22. Long, D. W. (1971) *Diss. Abstr. Inter.* **32**, 2035.
23. Rohrlich, M. and Essner, W. (1960) *Ber. Getreidechemiker-Tagung Detmold* 27–39 cited in *Chem. Abstr.* **55**, 10602e.
24. Pant, R. and Kapur, A. S. (1963) *Naturwissenchaften* **50**, 95.
25. Quillet, M. and Bourdon, D. (1956) *Compt. Rend.* **242**, 1054.
26. Phillips, D. V. and Smith, A. E. (1974) *Can. J. Botany* **52**, 2447.
27. Vasilevskaya, V. K. and Ya, E. (1970) *Vestn. Leningrad Univ. Biol.* **1**, 33 cited in *Chem. Abstr.* **73**, 53043e.
28. Takiura, K. and Nakagawa, I. (1963) *Yakugaka Zasshi* **83**, 298 cited in *Chem. Abstr.* **59**, 8849b.
29. Bailey, R. W. (1963) *Nature* **199**, 1291.