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THE DISTRIBUTION OF ANTHRAQUINONE GLYCOSIDES IN *CASSIA SENNA* L.

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Abstract—The distribution of rhein-type and aloe-emodin type anthraquinone glycosides in developing *Cassia senna* L. plants has been studied. The seeds contain no anthraquinones but shortly after germination chrysophanol, then aloe-emodin and finally rhein are formed in the young plant. In the presence of adequate light, glycosylation follows and significant quantities of glycosides appear in the young leaves. These are probably translocated to the flowers and ovaries since large concentrations of glycosides accumulate there. During fruit development the amount of aloe-emodin glycoside in the pericarp falls when the seeds become separable from the pericarp and the amount of rhein glycoside falls markedly when the seeds mature and become viable. It is suggested that in this plant anthraquinone production is intimately associated with fruit and seed development.

INTRODUCTION

THE almost mature fruits of *Cassia senna* L. contain up to 4 per cent of anthraquinone glycosides calculated as sennosides A and B.[1] These glycosides are contained in the pericarp only, which represents about half the weight of the fruit, and therefore must contain up to about 8 per cent of these compounds. Furthermore, if the glycosides are present mainly in the form of the sugar-rich primary glycosides,[2] it may well be that the pericarp contains about 15 per cent of anthraquinone compounds calculated as primary glycosides. This seems a significant proportion of substance and we therefore decided to study the distribution of these glycosides in the developing leaves, fruits and seedlings as a contribution to our knowledge of their possible function in the plant. Little work has been done on this problem; Saber, Balbaa and Award[3] showed that the glycosides of the leaf gradually increased to a maximum at the flowering stage and then decreased as the fruits developed. Work on other anthraquinone-containing plants indicates that in *Rheum* species the quantities in the leaf are related to photosynthetic activity[4] or to the location within the leaf and its age.[5] In *Rhamnus* species there is some evidence that the anthraquinone glycosides are stored in the bark during the winter and used in the spring for the rapid development of the shoot and leaf.[6,7] In *Aloe* species the content of aloin varies monthly with a maximum during the summer months.[8]

In the following account the glycosides of senna are classified for convenience as (*a*) *rhein-type*, characterized by the presence of carboxylic groups in the aglycone. The best-known members of this group are the sennosides[9] but sugar-rich *primary glycosides* which break down

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to sennosides during storage and extraction may also be present.[2] (*b*) *Non-rhein glycosides* contain no carboxylic group and are based mainly on aloe-emodin.[10] The two groups of glycosides can be estimated separately after hydrolysis, by separating the aglycones by distribution between ether and $NaHCO_3$ solution. The heterodianthrones[11,12] would be included with the rhein-type glycosides by this method.

RESULTS

Work on the Leaf

The experiments of Saber, Balbaa and Award[3] were made on presumably mature leaves collected at different stages of growth of the *plant*. In contrast we analysed samples representing different stages of growth of the *leaf* from plants at the same stage of growth. The results are recorded in Table 1. In view of the striking decrease in glycosides and increase in aglycones in the Growth Chamber plants, the water-soluble carbohydrates of both types of plants were determined. In the Greenhouse plants 10·0–10·6 per cent was present (calculated as glucose) but in the Growth Chamber plants only 7·0–7·2 per cent.

TABLE 1. ANALYSES OF LEAVES AT DIFFERENT STAGES OF DEVELOPMENT FROM (*a*) 9-MONTH OLD GREENHOUSE PLANTS WITH NORMAL LIGHTING, AND (*b*) 6-MONTH OLD GROWTH CHAMBER PLANTS WITH LOW LIGHT INTENSITY

(Glycosides and free anthraquinones all calculated as sennosides)

Stage of development	Average dry weight of 1 leaf (mg)	Glycosides (%)		Free anthraquinones (%)
		Rhein-type	Non-rhein type	
Greenhouse				
(*a*) Unexpanded leaves on young shoots	0·90	6·76	0·75	1·20
(*b*) Expanded small leaves on young shoots	1·94	4·80	0·53	Traces
(*c*) Fully expanded leaves on young shoots	6·15	2·91	0·20	Traces
(*d*) Fully expanded leaves on main stem	5·00	2·50	0·10	Traces
Growth Chamber				
(*a*) Unexpanded leaves on young shoots	0·41	4·82	0·83	1·20
(*b*) Expanded small leaves on young shoots	1·66	2·75	0·62	0·62
(*c*) Expanded large leaves on young shoots	6·08	0·25	0·0	0·41
(*d*) Expanded large leaves on middle of stem	6·80	0·25	0·0	0·50
(*e*) Expanded large leaves at bottom of stem	8·91	Traces	0·0	1·10

Work on the Fruit

Commercial samples of senna pod contain fruits varying from pale greenish brown, when immature, to dark-brown ripe pods. Analysis of the pale pods showed a high content of glycosides (3·0–4·3 per cent calculated as sennosides) in contrast with the dark pods which contained only 0·6–0·7 per cent. Of considerable interest was the fact that the germination rate of the seeds from the pale pods was practically zero whereas from the dark pods 80–90 per cent of the seeds germinated within a few days of sowing. It was therefore decided to examine pods and seeds at known stages of development. Senna plants produced racemes each with flowers and fruits of varying age so that it is necessary to handle a very large number of racemes in

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TABLE 2. ANALYSES OF DEVELOPING SENNA FRUIT COLLECTED AT WEEKLY INTERVALS. (Glycosides and free anthraquinones all calculated as sennosides)

Week collected	Fruits		Rhein-type glycosides		Non-rhein type glycosides		Free anthraquinones		Seeds
	Average wt. (mg)	Proportion of seeds (% w/w)	% in pericarp*	Absolute amt. per fruit (mg)	% in pericarp	Absolute amt. per fruit (mg)	% in pericarp	Absolute amt. per fruit (mg)	Germination rate (%)
0 Flowers	8·0	—	2·63	0·20	0·26	0·02	0·18	0·14	—
0 Ovaries	1·0	—	8·93	0·09	0·72	0·01	0·40	0·01	—
1	3·0	—	9·34	0·28	1·65	0·05	0·61	0·02	—
2	5·3	—	10·26	0·55	1·89	0·10	1·05	0·06	—
3	11·7	—	9·13	1·07	1·65	0·19	0·91	0·11	—
4	18·7	—	9·75	1·83	1·73	0·32	0·71	0·13	—
5	24·6	—	9·22	2·26	1·45	0·36	0·43	0·11	—
6	31·5	—	7·50	2·36	1·20	0·38	0·58	0·18	—
7	41·6	2·0	5·71	2·33	0·76	0·31	0·25	0·10	0
8	62·6	13·0	4·82	2·64	0·33	0·21	0·23	0·13	0
9	69·8	12·0	4·74	2·91	0·34	0·21	0·15	0·10	0
10	76·9	13·0	4·70	3·15	0·27	0·18	0·17	0·12	0
11	92·6	13·2	4·35	3·52	0·23	0·18	0·18	0·14	0
12	128·9	36·1	4·01	3·30	0·23	0·20	0·13	0·10	16
13	206·4	62·9	3·90	3·00	0·21	0·17	0·21	0·17	76
14	195·0	66·1	3·57	2·36	0·15	0·10	0·74	0·49	96

* Up to week 6 includes immature seeds which could not be separated from the pericarp.

order to get suitable samples. However attempts to produce sufficient plants up to fruiting stages under artificial conditions in England were unsuccessful. A preliminary experiment, therefore, on samples collected at known stages from plants growing in the desert near Omdurman, Sudan, was carried out, but owing to damage to the plants by grazing goats and camels it was not possible to collect a complete set of samples. Analysis of those collected, however, confirmed the fact that very young fruits contained high percentages of glycosides (up to 10 per cent calculated as sennosides) and as the fruits matured the percentage fell but the germination rate of the seeds increased. A second experiment was attempted on plants growing in the Botanical Garden of the Faculty of Pharmacy, Cairo. Several hundred racemes, whose lower buds were just opened, were labelled with tags. Samples of fifty opened flowers were collected at this stage (week "0") and similar samples collected from the same positions on the racemes at weekly intervals till mature fruits were formed. The samples were dried in the sun, sent to London, powdered and analysed for glycosidal content: the germination rates of the seeds at appropriate stages were also determined and the results recorded in Table 2.

Work on Seedlings

Seeds were germinated in vermiculite and the seedlings produced examined at various stages. Only small amounts of anthraquinone were produced so that a qualitative examination only was attempted. Chrysophanol and aloe-emodin, in the quinone form, were found to occur in the early stages; rhein only appeared after the seedlings had produced their own leaves. No emodin was found at any stage.

DISCUSSION

The most striking fact established by this work is the very high proportion of anthraquinone glycosides found in the ovary and young pericarp. Assuming these glycosides occur mainly as sugar-rich primary glycosides,[2] then up to 20 per cent or more is present at these stages. From about week 6 onwards, when the seeds become separable from the pericarp and increase in weight, the porportion of glycosides falls (though not the absolute amount in view of the continuous growth of the pericarp itself). During the last three weeks the seeds rapidly increase in viability and it is during this period that the absolute amount of glycosides falls markedly, by about one-third. Another interesting fact is the rapid fall in the relative amount of non-rhein glycosides about week 6. Up to then they represent about 15 per cent of the total glycosides: afterwards the proportion falls to about 5 per cent.

No anthraquinone compounds occur in the seed but shortly after germination chrysophanol (R—CH_3, where R = 1:8 dihydroxyanthraquinone) then aloe-emodin (R—CH_2OH) and finally rhein (R—CO_2H) are formed. This order is consistent with what is known of the biosynthesis of anthraquinones via the acetate-malonate route; the fact that rhein compounds soon predominate indicates that a strong oxidizing system is associated with the process. As would also be expected glycosylation appears to follow the formation of the aglycone; Table 1 shows that in fact when sugar production is reduced, glycoside content falls and free aglycones accumulate. Under normal conditions very high proportions of glycosides are formed in the young leaves and this amount falls as the leaves mature.

A tentative picture of the situation would be that, shortly after photosynthetic activity begins in the seedling, anthraquinones and their glycosides are formed. These accumulate rapidly in the developing leaf and are then possibly translocated to the developing ovaries and fruits. Here they accumulate in comparatively high concentrations but become depleted as

the seeds develop, especially at the later stages. A further interesting correlation is the marked drop in the relative proportion of non-rhein to rhein glycosides at the stage when the developing seeds become separable from the pericarp. These suggestions point to the fact that anthraquinone production in this plant is intimately associated with fruit and seed development.

EXPERIMENTAL

Plant Material

All plant material used was authenticated by reference to Herbarium speciments at Kew and by determination of seed characters and certain microscopical constants.[13]

Growth Conditions

Seeds were germinated in moistened vermiculite in the Growth Chamber and after the third pair of leaves had formed were transplanted to pots containing potting compost.

(*a*) *Growth chamber.* Illumination by fluorescent lamps giving 440 lm/ft^2 at plant level for 12 hr/day. Max. temp. 90°F; min. temp. 68°F.

(*b*) *Greenhouse.* Illumination by daylight supplemented by artificial lighting giving 2300–2500 lm/ft^2 at plant level for 14–15 hr/day. Max. temp. 96°F; min. temp. 50–60°F.

Analysis and Chromatography

Quantitative estimation of the anthraquinone glycosides was carried out by the Recommended Method for Senna[14] and of the water-soluble carbohydrates by the method of Nalewaja and Smith.[15] Paper chromatographic examination of the anthraquinones was by the method of Betts, Fairbairn and Mital.[16]

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