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the enamel-dentine interface, but the saline-enamel interface and the dentine-pulp interface can be seen. There is a fuzziness of the interproximal areas. With better resolution it should be possible to detect caries in the very earliest stages.

In order to improve the resolution, a higher ultrasonic frequency is required. It may be necessary to utilize frequencies in the 30 Mcy/sec region or higher. Although the coefficient of absorption of sound is high at 15 Mcy/sec, and doubles at 30 Mcy/sec, the latter frequency may be required, for the reason given. Since the carious process produces alterations in tissue, an acoustic interface is established. Ultrasound is capable of revealing differences in acoustic impedance of from 0.4 percent at -40 db to 0.004 percent at -80 db (3).

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Glucosides of Coumarinic and *o*-Coumaric Acids in the Tonka Bean

Abstract. The β -glucosides of coumarinic and *o*-coumaric acids were detected in extracts of cotyledons, exocarps, and seedling leaves of the tonka bean. The existence of these compounds and the presence of a β -glucosidase having specificity for coumarinyl glucoside suggest that the tonka bean synthesizes coumarin by a pathway similar to the one found in sweetclover.

Seeds of the tonka bean (*Dipteryx odorata* Willd.) are recognized as a rich source of coumarin (1). In 1940 Lutzmann (2) studied seeds of this species to determine whether coumarin occurs in nature as the β -glucoside of cou-

Table 1. Contents of coumarinic and *o*-coumaric acids in various tissues of the tonka bean.

Tissue	No. of extracts analyzed	Range in content (% of dry wt.)			
		Coumarinic acid		<i>o</i> -Coumaric acid	
		Free*	Bound	Free	Bound
<i>Immature fruit</i>					
Exocarp	2	0.01 to 0.02	0.01 to 0.10	0	0.39 to 0.44
Cotyledon	4	1.12 to 8.99	0 to 4.00	0	0 to 0.17
<i>Mature fruit</i>					
Cotyledon	5	0.62 to 6.15	0.06 to 6.79	0	0.11 to 0.23
<i>Seedling leaves</i>					
First†	2	0.11 to 0.17	2.10 to 2.80	0	2.14 to 2.25
Third†	2	0.15 to 0.28	2.12 to 2.23	0	0.45 to 0.65
Fifth†	1	0.27	0.81	0	0.15

* The lactone, coumarin, rather than the free acid, was actually present in the tissue extracts.
† First, third, and fifth leaves above the cotyledons were sampled as the respective leaves reached the fully unfolded stage.

marinic acid (*cis*-*o*-hydroxycinnamic acid). He concluded that no coumarinyl glucoside was present. In recent work, the β -glucosides of both coumarinic acid and *o*-coumaric acid (*trans*-*o*-hydroxycinnamic acid) were implicated as coumarin precursors in sweetclover (*Melilotus alba* Desr.) (3, 4) and possibly in sweetgrass [*Hierochloa odorata* (L.) Beauv.] (5), and the "bound coumarin" of sweetclover was identified as coumarinyl glucoside (6, 7). Although the presence of glycosidically bound coumarinic and *o*-coumaric acids in tonka bean leaves may be inferred from the recent studies of Griffiths (8), the occurrence of these glycosides remains uncertain because the paper contains information only on acid-hydrolyzed leaf extracts. We now present evidence indicating that the β -glucosides of coumarinic and *o*-coumaric acids exist in seedling leaves and also in the fruit of the tonka bean.

Three tonka bean seedlings were grown in soil under standard cool white fluorescent lights in the laboratory. Aqueous extracts of seedling leaves, various portions of immature fruits, and cotyledons of mature fruits were prepared by dropping the respective tissue samples into boiling water and immediately autoclaving for 20 minutes. This procedure minimized the action of endogenous glucosidases during extraction. Aliquots of the extracts were assayed fluorometrically for free coumarinic and *o*-coumaric acids, and other aliquots were hydrolyzed by autoclaving for 45 minutes in 2.25*N* NaOH before assay, to provide estimates of total levels of the two acids. Values for the bound compounds were calculated by difference. The assay procedure was essentially the one used in studies on sweetclover (9).

As shown in Table 1, the *cis* isomer of *o*-hydroxycinnamic acid predomi-

nated in cotyledons of both immature and mature fruits, but in the exocarp of the immature fruits the *trans* isomer was predominant. Less pronounced differences between levels of the two isomers were observed in seedling-leaf extracts. The *trans* isomer was not detected in the free form in any of the extracts, but both free coumarin (the lactone form of coumarinic acid) and bound coumarinic acid were abundant in extracts of cotyledons and seedling leaves. Extracts obtained from immature cotyledons within a few days after harvest of the fruits were comparatively low in content of free coumarin and rich in bound coumarinic acid. Similarly, extracts of cotyledons from fruits which apparently matured naturally were relatively low in free coumarin. However, extracts of cotyledons from immature fruits stored under refrigeration for several weeks, or from dried fruits which appeared to have been harvested before full maturity, were high in content of free coumarin. These observations suggest the possibility that the presence of large amounts of free coumarin may be an artifact resulting from the extraction procedure or from unnatural ripening or storage conditions. Similar findings regarding the presence of free coumarin in sweetclover were recently reported (7, 10).

In the tonka bean fruits, coumarinic and *o*-coumaric acids were not confined to the exocarp and cotyledons. However, interfering fluorescent substances were present in aqueous extracts of the mesocarp and endocarp, and available data on these parts are less reliable than the data presented in the table.

In chromatographic tests employing the four solvents described by Kosuge (6), R_F values observed for the bound forms of coumarinic acid and *o*-coumaric acid in tonka bean extracts were identical to values for authentic

samples of the β -glucosides of coumarinic acid and *o*-coumaric acid, respectively. The action of a preparation of β -glucosidase from almonds (Mann Research Laboratories) on the bound compounds furnished further evidence that the compounds were β -glucosides. Both bound compounds were hydrolyzed by the almond preparation; and, in agreement with reports of other workers (3, 5), the *trans* compound was hydrolyzed more rapidly than the *cis* isomer.

An active β -glucosidase was detected in extracts of tonka bean leaves and cotyledons. Unlike the almond β -glucosidase, however, the tonka bean enzyme rapidly hydrolyzed coumarinyl glucoside and was essentially inert toward *o*-coumarinyl glucoside. In its specificity for the *cis* glucoside, the tonka bean enzyme resembled sweetclover β -glucosidase (3).

The occurrence of the β -glucosides of coumarinic acid and *o*-coumaric acid and the presence of a glucosidase specific for the *cis* glucoside indicate that in the tonka bean, as in sweetclover (3), coumarin is synthesized through the conversion of *o*-coumarinyl glucoside to coumarinyl glucoside which under suitable conditions may be hydrolyzed to yield coumarinic acid. Spontaneous lactonization of coumarinic acid then produces coumarin (11).

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"Imprinting" in Nature

Abstract. *Much laboratory research is based on the assumption that the learning of parental characteristics by young nidifugous birds is rooted on visual factors. Naturalistic observations of ground- and hole-nesting duck species, augmented by sound amplification equipment, indicate that hatchlings are exposed to the call of their mother for a relatively long period before they are exposed to the sight of her.*

Considering the fact that ethologists have (almost?) returned the naturalistic observation of behavior to its former place of respectability, it was surprising for me to learn that Lorenz's formulation of imprinting was not based on comprehensive natural observation (1). Rather, he appears to have relied almost solely on his own and O. Heinroth's previous observations concerning the following-response of ducklings and goslings hatched in incubators. (Such birds tend to follow humans or the first relatively large moving objects which they see shortly after hatching.) Relying on these and other non-naturalistic observations, Lorenz delineated what he believed to be the major characteristics of imprinting, and it is indeed a tribute to his genius that much of what he described or inferred has been subsequently supported by laboratory research.

With an interest in determining the sensory environment which young precocial (nidifugous) birds experience upon hatching, the length of stay in the nest, and the hen's behavior in enticing the young to leave the nest, I embarked several years ago on naturalistic studies of hole-nesting and ground-nesting duck species. At the time I viewed this venture from the standpoint of only furthering my personal knowledge, but some of the results of these studies seem important enough to warrant public mention.

Since the time of Audobon, naturalists have recurrently observed the dramatic exodus of hole-nesting wood ducklings (*Aix sponsa*) from dark cavities located in trees some 20 to 60 feet above the ground (or water). In response to the hen's call, the ducklings ascend the vertical surface of the cavity, pause momentarily in the exit leading to the outer world, and then leap into space with nonfunctional wings aflutter, thereby joining their parent on the ground or water below.

Naturalists are accustomed to viewing avian behavior in terms of innate or instinctive predispositions, and it has been tacitly assumed that wood ducklings are innately responsive to the exodus call of the wood duck hen (2)—that is, the ducklings' responsiveness to the hen's call has been traditionally viewed as occurring relatively independent of previous experience, being determined by a largely preformed, inherited sensorimotor linkage. Though I personally do not understand this point of view any better than I understand the view that behavior is almost solely a function of experience or learning, I was still surprised by what we found concerning the sensory environment of wood ducklings during and after hatching. In the three instances where we were able to institute sound recording while the eggs were in the early pipping stage, we found the hen uttering a low intensity call ("kuk, kuk, kuk . . .") which she continued with rising amplitude and rate up to and including the exodus some 20 to 36 hours later. Thus there was ample opportunity for the ducklings to associate the hen's call with her presence for a prolonged period prior to the exodus. These observations allow the wood ducklings' responsiveness to the hen's call to be placed, however vaguely, in the category of associative learning. The proximity of the familiar stimulative source (the hen) presumably acts as the rewarding aspect for the ducklings (3).

The fact that auditory stimulation seems to play such an important role in at least the initial stages of "imprinting" in hole-nesting ducklings suggested that we ought to undertake comparative observation of a ground-nesting species. By virtue of differences in nest-site ecology, it has been suggested that visual factors may be more primary than auditory factors in the imprinting of ground-nesting ducklings. Though my wife and I have been able to monitor only one nest of a ground-nesting species (*Anas platyrhynchos*) thus far, I am inclined to mention our findings because they parallel those made by Robert I. Smith on one nest of another ground-nesting species (*Anas acuta*) (4). In both instances, while perched on the nest, the hens vocalized during and after the hatching of the young. In the case of *Anas platyrhynchos* the rate and intensity of the call gradually increased up to and including the exodus. Smith's recording