cycle was repeated (cycle No. 2) on the same sample. The irreversible change which resulted from the first treatment apparently lowered the glass transition temperature about 100°C. Cooling and repeating the cycle (Nos. 3 and 4) caused the glass transition temperature to rise somewhat. At the end of cycle number 4, the temperature was held at 460°C for 15 hours. The thermoset nature of the product is apparent from the next treatment (cycle No. 5). The sample was then cooled to 400°C, held at 400°C for 15 hours, cooled to 100°C, so that cycle No. 6 demonstrates the effect of further cross-linking.

Analysis in terms of the relative-rigidity and damping-index parameters provides a picture of the overall molecular changes which occur. The change from thermoplastic polymer to thermoset resin at about 450°C occurs with an intermediate process, possibly independent of the cross-linking, in which the thermoplastic backbone of the polymer is made flexible. Parallel analysis by infrared spectroscopy demonstrated that during a treatment represented by the first curve, the nature of the bonding of the hydrogen atom which is linked to nitrogen in the imidazole ring is altered drastically. Reduction of hydrogen bonding could account for the initial increase in mechanical flexibility. Subsequent chemical changes which accompany the cross-linking process are much less obvious. Cross-linking in the polybenzimidazole is accompanied by increasing rigidity in the rubbery region, a progressive increase in the glass transition temperature, and decreased damping in the thermosetting rubber (*5*).

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4. The polybenzimidazole was supplied by Dr. G. F. L. Ehlers of the Aeronautical Systems Development Laboratories, Dayton, Ohio.
5. I am indebted to Armand F. Lewis for an analysis of the method for estimating the damping characteristics and to Norman B. Colthup for advice on the interpretation of the infrared analyses.

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Observation of Internal Structures of Teeth by Ultrasonography

Abstract. *Preliminary experiments with high-resolution intensity-modulated ultrasonography techniques developed for ophthalmological diagnosis have demonstrated that at a frequency of 15 megacycles per second some internal structures can be seen in vital teeth. A relationship between time, tooth vitality, and ultrasonic viewing was observed.*

Some aspects of diagnostic ultrasonography warrant emphasis here. The intensity of ultrasound used in diagnostic ultrasonography has been shown to have no immediate, delayed, or cumulative injurious effect on tissue (*1*). Ultrasonography yields cross-sectional views without the superimposition of structure characteristic of x-rays. The observed increase in ultrasonic absorption with loss of tooth vitality forms the basis for a sensitive method of measuring viability.

Fifty pilot dental studies were made, with ultrasound provided by the General Precision Laboratories' ophthalmic ultrasonoscope, designed by Baum and Greenwood (*2*). Results of two of these studies are shown in Fig. 1. These demonstrate that internal viewing of tooth structure has been accomplished, although not with the desired clarity.

Studies were then made of freshly extracted teeth and of teeth extracted 1½ hours, 3 hours, and 5 weeks prior to the study. It was observed that as the time after extraction increased, the clarity with which the internal structure was revealed decreased. The internal structure of teeth extracted more than 3 hours prior to the study could not be seen. It appears that a relationship exists between the vitality of the tooth and the degree to which the internal structure is revealed by ultrasonography.

In the pilot studies frequencies of 15 Mcy/sec were used (Fig. 1). It may be observed that the resolution is not sufficient for differentiation of

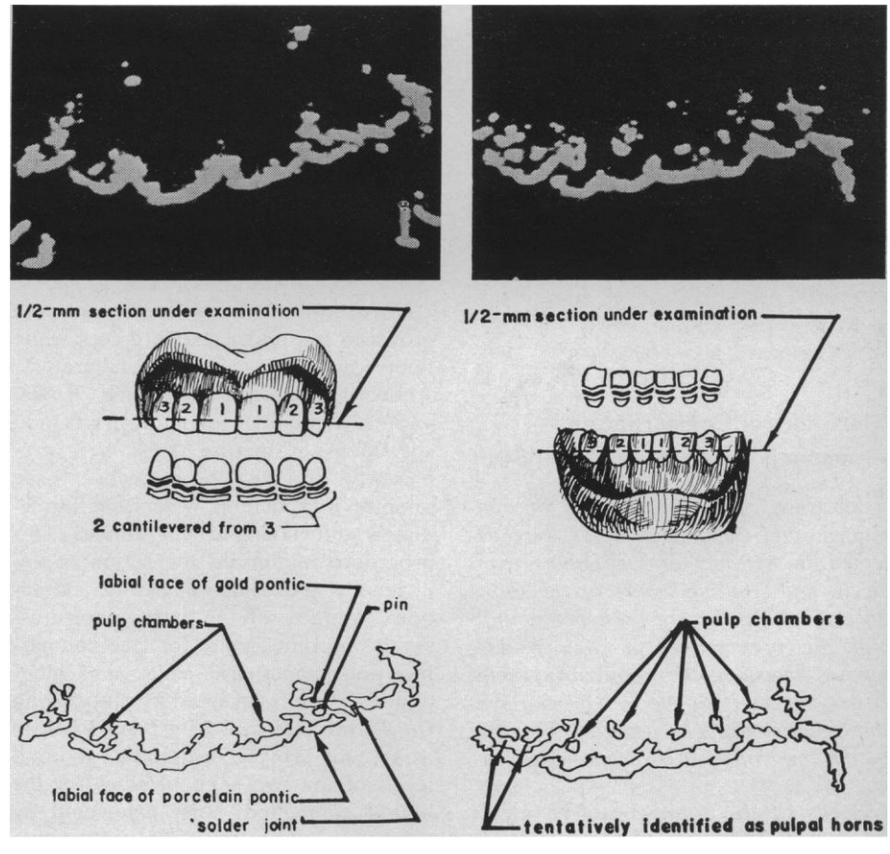

Fig. 1. Cross-sectional views obtained with ultrasound, and explanatory schematic drawings. The pulp chambers of the teeth do not appear in the correct anatomical positions because the ultrasonic energy is not normal to the tooth. The resultant echo is transposed away from the proper position. This is a result of using ophthalmological equipment for dental work.

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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4 December 1962

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 coumarinic 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 [*Hierochloe 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.

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		*o*-Coumaric acid	
		Free*	Bound	Free	Bound
		Immature fruit			
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
		Mature fruit			
Cotyledon	5	0.62 to 6.15	0.06 to 6.79	0	0.11 to 0.23
		Seedling leaves			
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.

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 predominated 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