MS may help to resolve controversies generated in the last few years regarding the exact amount and range of melatonin secretion in humans and regarding the nature of (i) pulsatile secretion, (ii) extrapineal sources (such as brain, peripheral nerve, harderian gland, retina, gastrointestinal tract), and (iii) urinary excretion.

Although the negative CI GC-MS technique is practicable for routine analyses of plasma (120 analyses per week), RIA's offer somewhat greater efficiency for analysis of larger numbers of samples. The RIA techniques do not require expensive equipment, and they provide a convenient means of assay that could be used for routine analyses; GC-MS could be reserved for validation of antiserum specificity and extraction procedures. In addition, the high sensitivity and selectivity attainable with negative CI GC-MS offers increased opportunity for detection and quantification of other trace organic compounds in a biological matrix at concentrations of parts per trillion.

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Cellulose to Sugars: New Path Gives Quantitative Yield

Abstract. *Cellulosic residues that had been treated with a small amount of chemical solvent under room conditions were quantitatively saccharified on enzyme hydrolysis. This treatment can be used to obtain simple sugars for the production of alcohol and other chemicals.*

We have found a way to obtain quantitative yields of glucose from the alpha cellulose in agricultural residues. Cornstalks, bagasse, alfalfa, tall fescue, and orchard grass pretreated with an organic solvent give up to 99 percent glucose conversions on hydrolysis by cellulase enzymes from *Trichoderma reesei* (formerly *T. viride*).

The saccharification of cellulosics has been studied for 90 years (*1*, *2*). To date, cellulose hydrolysis has been accomplished by using either acids or enzymes (*2*–*5*). However, sulfuric acid promotes the formation of undesirable sugar side products (*2*). This problem does not exist when cellulose is hydrolyzed by the multicomponent enzyme system known as cellulase. This system hydrolyzes cellulose, a high polymer of glucose, to cellodextrins, which are water-soluble polymers with a degree of polymerization of < 6, and to glucose (*3*–*5*) without the formation of other, undesirable side products. Cellulase from *Trichoderma reesei* is of practical interest since recent pilot studies and the development of a hyperproducing mutant indicate that commercial production of this enzyme is feasible (*6*–*8*). A drawback to the use of cellulases has been their inability to rapidly and totally degrade native cellulose (*9*). The crystalline structure of cellulose and the lignin that physically seals the surrounding cellulose fibers make enzyme hydrolysis difficult by protecting the cellulose from contact with the enzyme.

To quickly and accurately quantify cellulose in various samples of cellulosic residues, we wished to develop an analysis that would involve (i) extraction of the cellulose from the sample, (ii) hydrolysis of the cellulose by either an enzyme or an acid, and (iii) calculation of the cellulose content from the hydrolysis kinetics. An extensive literature survey was undertaken to screen for cellulose solvents suitable for this purpose. Four major categories of solvents were uncovered: strong mineral acids (*10*); quaternary ammonium (Triton) bases (benzyltrimethylammonium hydroxides) (*11*); aprotic solvents (dimethyl sulfoxide–paraformaldehyde, nitrosylics, sulfur oxides, and oxychlorides) (*12*); and metal complexes such as cupriethylene diamine, cadoxen, and zincoxen (*13*). Although these solvents have been used for viscometric studies of cellulose (*14*), the combination of solvent pretreatment and enzyme hydrolysis appeared to be a new concept.

A combination of intuition and trial and error resulted in the choice of cadoxen as a solvent. Cadoxen is a colorless, odorless solvent made by dissolving 5 percent cadmium oxide in 28 percent aqueous ethylenediamine. Procedures for making the solvent and its properties have been described (*15*–*17*).

The application of cadoxen in an analytical procedure worked well. Cellulose from various samples was extracted in excess cadoxen and hydrolyzed. The hydrolysis gave quantitative conversion of cellulose to sugars. Thus, the cellulose content could be directly calculated from the quantity of sugar formed without using complicated kinetic equations.

Success with the analytical procedure led to the development of a process that should be feasible on a commercial scale. In this process, particles of cellulosics in the size range 0.5 to 2 mm (or larger) are placed in small amounts of cadoxen at room temperature and let

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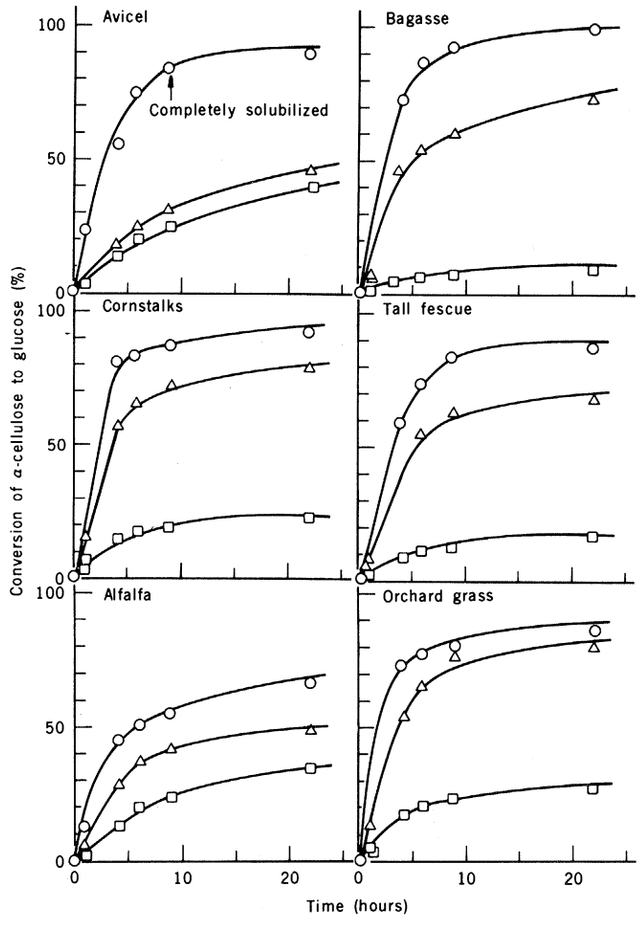

Fig. 1. Time course of enzymatic hydrolysis of cellulosic materials (○) treated with cadoxen, (△) treated with 28 percent ethylenediamine, and (□) untreated.

stand for 12 hours. Avicel, a pure microcrystalline cellulose, was treated at a solid:liquid ratio of 1:15. Agricultural materials that had not been otherwise treated (to remove hemicelluloses) were treated at a ratio of 1:5. These materials included cornstalks (35.6 percent alpha cellulose), bagasse (33.4 percent), alfalfa (24.3 percent), fall fescue (36.5 percent), and orchard grass (29.7 percent). The samples were analyzed for cellulose as described in (*18*).

Treated samples were washed with water to remove the solvent and added to buffered enzyme [*p*H 4.8, 8.4 I.U. per gram of residue (*19, 20*)]. These samples showed greatly enhanced rates and extents of saccharification compared to untreated samples when incubated at 45°C in a shaker bath. The amount of glucose formed was measured (*21*) and used to calculate the percentage of cellulose converted (*22*), with the results shown in Fig. 1.

Avicel was completely solubilized in 9 hours with 83 percent conversion to glucose. Low-pressure liquid chromatography (LPLC) (*23*) of Avicel hydrolysates showed that cellobiose, a dimer of glucose, was also formed (Fig. 2). Before solubilization (at 79 percent conversion to glucose and 88 percent total conversion), 9 percent of the cellulose converted to sugars was cellobiose (Fig. 2a). Some time after solubilization (94 percent conversion to glucose and 100 percent total conversion), 6 percent of the cellulose converted was still cellobiose. If enough time was allowed to pass (more than 48 hours), almost all of the cellulose converted to soluble sugars appeared as glucose (Fig. 2c). The slowness of conversion of cellobiose to glucose may be attributed to the inhibitory effect of the product glucose on the enzyme (*20, 24*). The data obtained by both the glucose and the LPLC analysis indicate that treatment with cadoxen disrupts the crystalline structure of cellulose and thereby renders it susceptible to rapid and total hydrolysis to soluble products.

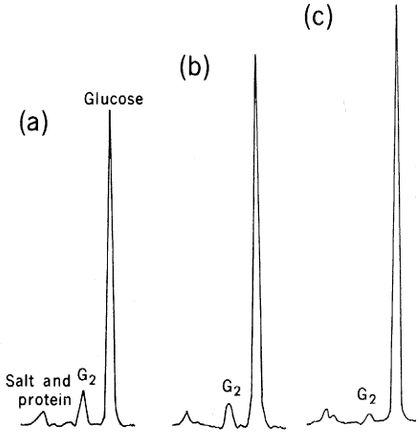

Fig. 2. Low-pressure liquid chromatograms of crystalline cellulose hydrolysates at (a) 79 percent, (b) 94 percent, and (c) 97 percent conversion to glucose; G_2 refers to cellobiose.

The experiments with the agricultural residues showed that solvent pretreatment is also effective for cellulose protected by a lignin seal. Treated bagasse, cornstalks, tall fescue, and orchard grass gave more than 90 percent conversion to glucose.

Agricultural residues treated with 28 percent ethylenediamine gave higher rates of hydrolysis and greater conversion to glucose than untreated materials. However, they were still lower than those for cadoxen-treated residues. Thus the action of this strong aqueous base alone is insufficient to produce the results observed for the cadoxen-treated materials.

Cadoxen has the further advantage that it can be recovered and reused. It can be washed completely from cellulosic materials with methanol and then water (*25*). Cadmium precipitates out as the hydroxide, which is insoluble in water. After filtration, cadmium hydroxide can be regenerated to the oxide form by heating to 250° to 350°C (*26*). Cadmium oxide can then be recycled to make more solvent. Ethylenediamine can also be recycled since it can be concentrated by evaporating off the excess wash water to give a 28 percent solution. Thus the use of a water-based solvent such as cadoxen is practical. Furthermore, the moisture levels normally found in agricultural residues do not appear to affect the solvent's potency. Only small quantities of the solvent are needed to obtain the desired effect.

The successful application of this solvent has prompted us and other researchers to develop analogs of cadoxen made from nontoxic components. The nontoxic solvents give results similar to those obtained with cadoxen (*27*). This should widen the range of applications for which solvent pretreatment of cellulosic residues might be suitable.

Utilization of biomass as a source of fuels, chemicals, and food is a complex problem requiring not only technical know-how but also "know-what, know-where, and know-when, and know-why" (*28*). The treatment described in this report can be used to obtain simple sugars for fermentation to alcohol and other chemicals. With nontoxic analogs it may be possible to treat corn stover to in-

crease its digestibility for cattle. In this area the combined efforts of chemists, biochemists, animal scientists, agronomists, agricultural and chemical engineers, and economists will be needed to develop more ways to utilize renewable resources for energy and food.

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Plant Chemistry and the Evolution of Host Specificity: New Evidence from *Heliconius* and *Passiflora*

Abstract. *Larval growth rates of* Heliconius *butterflies do not closely parallel host plant choice, an indication that factors other than host plant chemistry are important in evolving host specificity. High growth rate in one species is correlated with reduction in number of palatable host species. This suggests a mechanism by which ecologically restricted species become progressively biochemically specialized.*

Monophagy, defined at the local population level as the feeding of a consumer on just one species of host (*1*), has evolved repeatedly in herbivorous insects. It is particularly common among leaf-eating insects, and is thought to be principally a response to the great diversity of toxic secondary plant compounds found in the leaves of higher plants (*2–4*). While monophagy and plant compounds are undoubtedly related, our results indicate that monophagy may evolve initially as a result of "ecological" factors such as predation or plant abundance, rather than by differences in host palatability (*5*). The results also suggest that, once this type of monophagy is established, selection for increased digestive efficiency may cause the insect to slowly lose the ability to feed on its former host plants. When this happens the insect becomes sensitive to chemical barriers, which are believed to be so important in insect-host plant relationships (*6*). The proposed sequence of ecological monophagy followed by varying degrees of obligate monophagy may help to explain why a given insect group can vary widely in host specificity. It also provides a possible new mechanism for insect-host plant coevolution, the process proposed by Ehrlich and Raven, that results in parallel phylogenies between higher taxa of insects and their host plants (*3*).

In spite of the recognized importance of this phenomenon, the evolution of host specificity in insect-host plant interactions has received only minimal attention. One of the few general texts (*7*) dealing with the evolution of host plant choice concentrates on the effects of host plant chemistry and only very briefly mentions two other contributing factors: searching ability and competitive interactions. Yet, the importance of host plant chemistry remains to be critically evaluated, and other possible selective factors need to be assessed as well. At least three studies on butterfly species have indicated that chemically palatable but unused host species are available in the butterflies' habitat (*8*), which points out the inadequacy of host plant chemistry as being the sole determinant of host plant choice. Also, even when acceptable alternative hosts are not present, a satisfactory model is lacking as to how monophagy could evolve from less specialized ancestors (*3*, *9*).

I investigated this problem by studying oviposition behavior and larval growth ability in three sympatric species of *Heliconius* butterflies. The aim of the study was to determine which host plants were being used by the butterflies in nature and then to compare this with the ability of the larvae of these butterflies to grow on the various host plants. If larval growth ability were exactly parallel to host plant choice in the field, this would indicate that host plant specificity in these butterflies is being enforced by host plant chemistry. If, in contrast, host plant choice were much more restricted than larval growth ability, this would indicate that other factors were promoting host specificity in these butterflies. I also attempted to test the hypothesis that butterflies with host-specific larval growth ability (*10*) would have enhanced growth rate on their chosen host plant. This hypothesis is predicted if insects become host-specific in order to enhance digestive and growth efficiency on one host plant species (*11*, *12*).

Host plant choice was determined in the field by collecting eggs on the various species of *Passiflora* at the field site (*13*). *Passiflora* and related genera are the sole host plants for larvae of the genus *Heliconius* (*4*). The eggs and larvae collected were reared to determine their identity. The numbers in parentheses in Fig. 1 represent these data. Sample sizes are unavoidably small due to difficulties in locating eggs and larvae and also to difficulties in rearing for species identification. Therefore, these data were supplemented by a host plant choice experiment. Females of the three *Heliconius* species were tested. These were descended at least five generations from wild-caught *Heliconius* at the field site, except for the data from *H. erato* which

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