likely that the microorganisms first convert limonin to deoxylimonin, which is then transformed to deoxylimonic acid.

In earlier work Nomura (1966) reported that *Aspergillus niger* and two species of *Penicillium* were capable of producing enzymes which metabolize limonin and nomilin. Activities of these enzymes were, however, very low, and no metabolites were isolated. The results obtained in this study show clearly the existence of enzymes capable of catalyzing debittering reactions. More important, the fact that both of these metabolites are nonbitter is of considerable practical significance.

Further investigations on characterization of limonin metabolites, isolation, and characterization of the organisms, and isolation of enzymes involved in the metabolism of limonin are in progress.

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Characterization of the Major Aroma Constituent of the Fungus *Trichoderma viride* (Pers.)

The volatile constituents of *Trichoderma viride* (Pers.) were resolved by glc. The major compound was characterized as 6-pentyl-α-pyrone by ir, ms, and nmr spectroscopy, elemental analysis, and by hydrogenation to known derivatives. The characteristic coconut-like aroma produced by this fungus is certainly due to this compound.

A commonly occurring soil fungus (*Trichoderma viride*) has been extensively studied from the aspects of its saprophytic potential and because of its antagonism and parasitism against other fungi. The fungus produces in culture a characteristic coconut-like aroma and this report is concerned with the identification of the major constituent responsible for the characteristic odor.

The organism used in this study was isolated by the authors from a soil sample collected near Coventry Lake in Coventry, Conn. The organism was grown in a potato dextrose (10%) liquid medium containing $CaCO_3$ (0.2 g/l.) and $MgSO_4$–$7H_2O$ (0.2 g/l.) in quart milk bottles containing 60 ml of the medium. After inoculation, the milk bottles were placed on their side and incubated at room temperature for 3 to 4 days. At the end of this period the surface of the medium was covered with the dark green conidia characteristic of this organism.

At the end of the growth period the cells and culture medium were steam distilled at atmospheric pressure. For the analyses reported here approximately 18 l. of medium was distilled. The aqueous distillate was saturated with NaCl and then extracted with redistilled ether. The extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure, leaving 3.3 g of a yellow oil having a strong coconut-like aroma. The oil was separated into its components by glc (Hewlett-Packard Model 7620A) utilizing a flame ionization detector equipped with an effluent splitter. An 8 ft × 0.125 in. o.d. stainless steel column packed with carbowax 20M (10%) and phenyldiethanolamine (PDEAS 1%) on 80–100 mesh A.W. Chromosorb W was used. The temperature was programmed from 80–220° C at 6° C/min. The compounds were collected as they exited from the instrument by the procedure described by Halim and Collins (1970). A typical glc analysis is shown in Figure 1.

The major component of the oil (over 90%) had a retention time of approximately 24 min. The isolated compound was a colorless liquid having a strong coconut-like odor. This compound was further purified at 135° C in a 6 ft × 0.125 in. o.d. stainless steel column packed with W98 (10%) on 80–100 mesh Chromosorb W.

The infrared spectrum showed two C=O absorption bands at 1740 and 1725 cm^{-1} and two strong C=C stretching bands at 1636 and 1553 cm^{-1}, suggesting an α-pyrone ring (Nakanishi, 1962). Infrared spectra were taken as thin films contained between KBr disks (13 mm diam) on a Perkin–Elmer Model 137B Infracord Spectrometer.

The mass spectrum showed a parent ion at *m/e* 166. The molecular formula was assigned as $C_{10}H_{14}O_2$ on the basis of the mass spectral data and elemental analysis (Found: C, 72.84%; H, 8.39%; O, 18.77%; calcd: C, 72.9%; H, 8.50%; O, 19.21%). The mass spectrum also revealed a base ion at *m/e* 95 attributable to $M - C_5H_{11}$. Mass spectra were obtained on an M.S. 12 mass spectrometer using a probe inlet system, ion source, operated at 250° C.

The (nmr) showed signals at δ 0.94 (3 H, triplet), 1.4 (6 H,

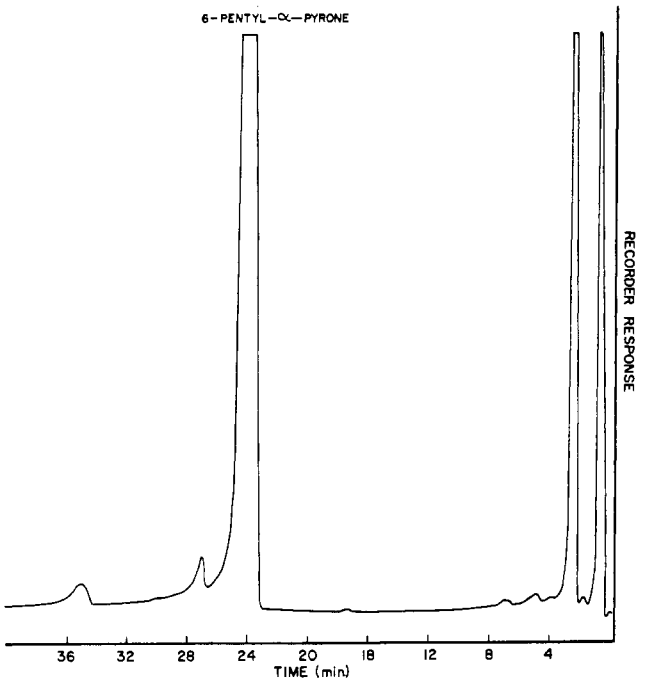
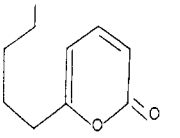


Figure 1. Gas chromatogram of volatiles from *Trichoderma viride* using a Carbowax 20M and PDEAS column

complex multiplet), and 2.48 (2 H, triplet) all consistent with an *n*-pentyl group. The three α-pyrone ring protons formed an ABX pattern with δ_A 5.83, δ_B 6.00, and δ_X 7.19 and J_{AX} = 6.5 and J_{BX} = 9.5 Hz. The ABX protons represent those attached to positions 5, 3, and 4, respectively. All nmr spectra were obtained on a Varian A-60 nuclear magnetic resonance spectrometer.

As an additional proof, the compound was hydrogenated over Adams platinum oxide in ethanol. When the reduction product was subjected to glc (10% W98 column) two peaks were revealed with an approximate peak area ratio of 1:5. The minor peak was identified as *n*-decanoic acid (infrared, retention time) and the major peak was identified as δ-decalactone (infrared, retention time, odor).

The structure of 6-pentyl-α-pyrone is here shown.



This is the first time, insofar as the authors are aware, that 6-pentyl-α-pyrone has been isolated from a fungus; it has been, however, very recently found in peach essence by Sevenants and Jennings (1971). Nobuhara (1969) has synthesized 6-pentyl-α-pyrone along with other unsaturated lactones and a comparison of his published (ir) spectrum with that of the compound reported here shows them to be identical.

It is of interest to note that the origin of this compound in the fungus is primarily the spore rather than the hyphae. This same situation was found by Gehrig and Knight (1958, 1963) in the case of the production of methyl ketones by *Penicillium roqueforti*. Finally it would seem that the yield of this compound by the mold might make it amenable to the flavor industry as a source of a new and interesting natural flavor constituent.

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Determination of Sulfur in Plant Material by Oxygen Flask Combustion

A method is described for the determination of sulfur in plant material. One gram of dry plant material is combusted in a 5-l, oxygen-filled flask. Sulfur is determined turbidimetrically as barium sulfate. Results of analyses of sulfur in plant materials of known sulfur content are presented. The precision of the method was determined (σ = 0.0097) by repeated analysis of one sample of Ladino clover.

The determination of sulfur in plant tissue is subject to error due to volatilization of reduced sulfur compounds during ashing. In open systems, a basic oxidizing substance such as sodium peroxide or basic magnesium nitrate must be added to prevent such losses. These procedures are tedious and must be carried out slowly to prevent ignition which makes the determination worthless. The amount of salt added can also lead to errors in the subsequent precipitation of barium sulfate. Open systems are also subject to errors due to sulfur absorption from flames if a gas burner is used for ashing or from the walls of furnaces routinely used for dry ashing of plants. A comprehensive literature survey on the subject of the determination of sulfur in agricultural samples has been prepared (Beaton *et al.*, 1968). The determination of sulfur in drugs employing an initial combustion in a closed 500-ml flask to obviate vaporization losses was first reported by Schoniger in 1956. Combustion of plant material using this same flask prior to the determin-