lished by the nmr. Protons H_h (δ 6.11) and H_i (δ 6.89) are coupled to each other (J = 1.5 Hz) and show long-range interactions with the vinyl methyl group ($J_{h,i}$ slightly $< J_{i,i}$ = 1.5 Hz). $J_{h,i}$ compares well with the reported $J_{4,5}$ of 2,5-dihydrofuran-2-one.[9] The existence of long-range coupling in unsaturated oxygen heterocyclic compounds has been previously documented.[10]

A broad peak at δ 4.90 is assigned to H_c (the allylic alcohol), an assignment confirmed by its disappearance upon oxidation of strigol to the ketone strigone (**1b**) [noncrystalline, m/e 344.127 ($C_{19}H_{20}O_6$ requires 344.126)] with MnO_2, 2,3-dichloro-5,6-dicyanoquinone, or acetic anhydride in dimethyl sulfoxide. The first two reagents give evidence for an allylic alcohol moiety, confirmed by increased intensities in the spectral bands of strigone at 234 nm (ϵ 27,200) and 1675 cm^{-1}, indicative of an α,β-unsaturated ketone. In strigone the geminal dimethyl nmr peaks are shifted to δ 1.27 and 1.30 (demonstrating proximity to the newly formed ketone) and H_f is also shifted downfield to 5.76.

Hydrogenation of strigol (Pd/C, ethyl acetate) yielded a hexahydro derivative [m/e 352.188 ($C_{19}H_{28}O_6$ requires 352.188)]. The base peak of this compound was due to $C_5H_7O_2$[6] suggesting that the $C_5H_5O_2$ fragment of strigol had taken up 1 mol of H_2, but still retained two sites of unsaturation.

The complete structure of strigol was determined by X-ray crystallographic analysis of a single crystal.

Strigol crystallizes from benzene–hexane as needles elongated along the crystallographic a axis. The crystals are orthorhombic, space group $P2_12_12_1$, with a = 9.15, b = 12.37, and c = 15.37 Å, and Z = 4. Intensity data were recorded by equiinclination multiple-film Weissenberg photography with Cu Kα radiation, and they were measured on a scanning microdensitometer. A 13-atom partial structure was obtained from an E map based on 218 E values > 1.0 for which phases were derived by application of the symbolic addition procedure[11] and tangent-formula refinement.[12] The remaining nonhydrogen atoms were located in two subsequent electron-density distributions computed with the observed structure amplitudes and calculated phases. Atomic positional and anisotropic thermal parameters were refined by full-matrix least-squares calculations to the present R of 0.108 for 1650 reflections. Individual bond lengths and valency angles agree well with accepted values. The shortest intermolecular separation at 2.79 Å involves the hydroxyl oxygen atom and a carbonyl oxygen atom, and it is indicative of hydrogen bonding. All other intermolecular separations exceed 3.0 Å and represent normal van der Waals' interactions. The absolute configuration of **1a** was not defined, but is under active investigation. Structure **1a** shows relative stereochemistry.

It follows from the above that the second stimulant isolated by us, strigyl acetate[2] [m/e 388.149 ($C_{21}H_{24}O_7$ requires mass 388.152)], has structure **1c**. It has previously been stated that the *Striga* and *Orobanche* stimulants are closely similar.[5b] We have found that the *Striga* stimulants from corn[5d] show similar chromatographic behavior to those from cotton. The extreme potency of strigol and the presence of similar stimulants in a variety of plants raise the possibility of a biological role in the producing plant and suggest that strigol may be a representative of a new class of plant hormones. Other biological effects of strigol have not been tested, but its structure is reminiscent of unsaturated lactones found by Kupchan to have cytotoxic and antitumor activity.[13]

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Metallocenes. A Novel Class of Reagents for Protein Modification. I. Maleic Anhydride–Iron Tetracarbonyl[1]

Sir:

Metallocenes have many desirable features for the study of both protein function and structure,[2] but have hardly been utilized for this purpose. We have employed maleic anhydride–iron tetracarbonyl (MAIT),[3] a metallocene-like compound, which combines the reactivity of maleic anhydride toward peptides and proteins[4] with the probe characteristics of a π-bonded, iron(0) carbonyl system (Figure 1). The strong absorption of its triply bonded carbonyls can be resolved in the mid-infrared spectra of proteins modified with this reagent.

The anhydride reactivity of MAIT closely resembles that of maleic anhydride, as evidenced by their comparable first-order rates of hydrolysis in 40% aqueous acetonitrile ($t_{1/2}$ = 1.0 and 2.2 min, respectively, apparent pH 7, 23°). Ribonuclease (RNase) was chosen to compare the reactivity of MAIT and maleic anhy-

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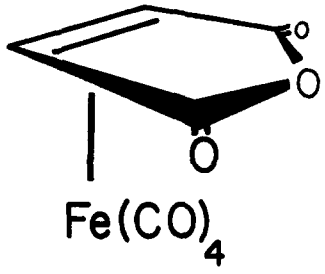

Figure 1. Maleic anhydride–iron tetracarbonyl.

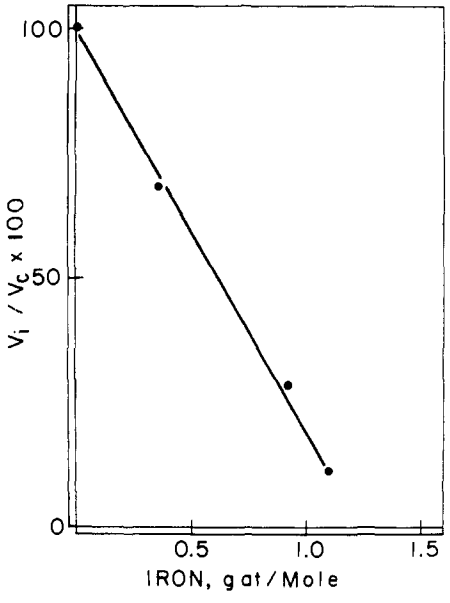

Figure 2. Reaction of MAIT with MA-RNase: progressive inactivation and iron incorporation after exposure to one-, three-, and fivefold molar excesses of MAIT for 1 hr, 23°, followed by filtration on Bio-Gel P-6, pH 1.2, 4°.

dride toward native proteins. Increasing molar excesses of either MAIT or maleic anhydride at pH 6 in 0.1 *M* 2-(*N*-morpholino)ethanesulfonic acid buffer, 2% acetonitrile, progressively inactivate RNase to the same extent, when cytidine cyclic phosphate is the substrate. Excess reagent and its degradation products are removed by gel filtration at pH 1.2, 4° for 5 min, conditions which we have found not to affect these modified enzymes. Reaction of RNase with a 12-fold molar excess of either MAIT or maleic anhydride at pH 6, 23° for 1 hr, yields yellow and white derivatives, respectively, crystallizable at pH 5. They exhibit from 2 to 6% of the activity of the native enzyme. Such MAIT-RNase contains nearly 4 g-atoms of Fe/mol of enzyme.

Both derivatives remain inactive when stored at pH 6 or 8, 23°, for 1 week, but gradually regain close to 90% of the control activity when kept at pH 2.9, 23°, for 1 week. Thus, covalent modifications of proteins with maleic anhydride[4] or MAIT are reversible at low pH and such derivatives are best investigated at neutral or alkaline pH. A sixfold molar excess of MAIT, while introducing 2.4 g-atoms of Fe/mol of RNase, decreases activity to only about 30%, a relatively nonspecific reaction.

Hence, RNase was labeled differentially. It was exposed first to a 12-fold molar excess of maleic anhydride, pH 6, in the presence of 0.01 *M* pyrophosphate, a competitive inhibitor.[5] This was followed by gel filtration

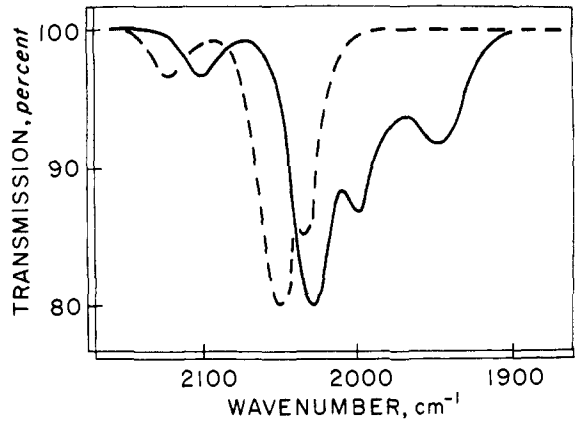

Figure 3. Infrared absorption of MAIT-MA-RNase (——) as a KBr pellet and of MAIT dissolved in $CHCl_3$ (– –).

at pH 8.2 in 10^{-3} *M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer. The resultant, inhibitor-free maleyl-RNase (MA-RNase) is fully active. On exposure of MA-RNase to a fivefold molar excess of MAIT, the product, MAIT-MA-RNase, exhibits 14% of the activity of the native enzyme and contains 1.1 g-atoms of Fe/mol of enzyme (Figure 2).

The difference absorption spectrum of MAIT-MA-RNase *vs.* MA-RNase exhibits a maximum at 290 nm (ϵ 8000), similar to that of MAIT in chloroform (λ_{max} 290 nm (ϵ 10,000)). Further, for MAIT-MA-RNase a Cotton effect with a negative extremum at 300 nm ($[\theta]_\lambda^{25}$ −5000°) is apparent, but it is absent for MA-RNase. The infrared spectrum of MAIT-MA-RNase displays absorption typical of vibrational stretch due to triply bonded carbonyls. Compared to the spectrum of maleic anhydride–iron tetracarbonyl, that of MAIT-MA-RNase undergoes an overall bathochromic shift of about 20 cm^{-1} and acquires a new band near 1950 cm^{-1} (Figure 3). The number and positions of these bands reflect the nature and arrangement of the bonds which contribute to metal carbonyl moieties.[3,6]

While MAIT was chosen as a prototype of metallocene-like compounds, a wide permutation of ring systems, metals, and ligands can generate analogous reagents encompassing a variety of spectral features which can be coupled to known, site-specific agents.[7] Such compounds are suggested both for spectral and crystallographic studies. The ability to react amino acid side chains of proteins with metallocenes of varying metal content should permit the site-specific introduction of metal atoms of widely variant probe properties and electron density. Studies along these lines are in progress.

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