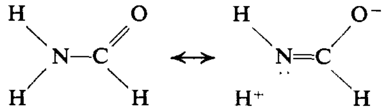
The H-bonded proton loses net charge on H-bond formation and Y and B both gain (B stands for *all* the rest of the atoms on the proton donor molecule).

Using the more complete variation in H-bond energy with O···N distance and C=O···N angle computed by Dreyfus and Pullman for linear formamide, we can conclude that a linear amide bond shorter than about 2.6 Å and longer than 3.4 Å is weaker than the completely optimized amide–water H bonds studied here. Thus, because of the geometrical constants imposed in carboxypeptidase,[1] some of its amide H bonds are weaker than optimum amide–water bonds.

These results should have considerable implications in the examination of secondary structure of proteins. In particular, model calculations of peptide conformation might now consider competitive amide–amide and amide–water H bonding in proteins; the H-bond energies found here should provide useful starting points for these potential functions.[12]

Also, by comparing the H-bonding ability of formamide, formaldehyde, and ammonia, we have provided the first semiquantitative estimate of the effect of the amide resonance structure



in increasing the H-bond base strength of a carbonyl group and the H-bond acid strength of an N–H bond.

Acknowledgments. One of us (P. A. K.) appreciates useful discussions with I. D. Kuntz.

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Germination Stimulants. II. The Structure of Strigol—A Potent Seed Germination Stimulant for Witchweed (*Striga lutea* Lour.)[1,2]

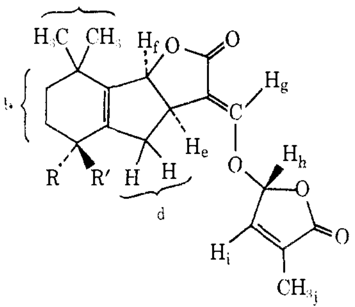
Sir:

We wish to report the structure of strigol (**1a**), a novel, highly potent seed germination stimulant isolated from the root exudates of cotton (*Gossypium hirsutum* L.).[2] In the absence of exogenous stimulant the seeds of the parasite witchweed (*Striga lutea* Lour.) do not germinate, but application of 10^{-11} *M* solutions of strigol results in over 50% germination.

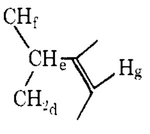
Witchweed is a chlorophyll-bearing parasite which attaches to the roots of corn and numerous other gramineous crops, causing severe damage. A native of the Eastern Hemisphere, witchweed was discovered in the United States in 1956 and is the object of a costly control program.[3] The demonstration that seeds of parasitic *Striga* species (and the related *Orobanche* parasites) require a chemical germination stimulant exuded from growing plant rootlets[4] led to numerous investigations of the nature of the stimulant or stimulants.[5] Until now, however, their structure has remained obscure.

Strigol [*m/e* 346.1408 ($C_{19}H_{22}O_6$ requires mass 346.1416), white needles from benzene-hexane, mp 200–202° dec] exhibited $\nu_{\max}^{CH_2Cl_2}$ indicative of OH (3590 cm^{-1}), butenolide (1787, 1745 cm^{-1}), and enol ether or unsaturated ketone (1682 cm^{-1}). Although a uv maximum at 234 nm (ϵ 17,700) was compatible with an unsaturated ketone, this moiety was ruled out by lack of a weak absorption band above 300 nm. The mass spectrum showed rapid cleavage to the fragments $C_5H_5O_2$[6] and $C_{14}H_{17}O_4$,[6] the latter undergoing facile dehydration to $C_{14}H_{15}O_3$.[6]

The nmr spectrum of strigol was highly characteristic, and in conjunction with spin decoupling experiments allowed the proton resonances to be unequivocally assigned. The geminal dimethyl groups (H_a) appear as singlets at δ 1.16 and 1.08,[7] the four cyclohexane methylene protons (H_b) at 1.52 (br), and the vinylic methyl (H_j) at 1.99 (t, J = 1.5 Hz). The cyclopentane methylene hydrogens (δ 2.67) (H_d, d) are essentially equivalent, but are coupled to H_e (J = 6 Hz). The latter resonance (δ 3.61) is also coupled to H_f (δ 5.48 (d, J = 8 Hz)), and allylically coupled to the highly deshielded proton H_g (δ 7.42 (d, J = 2.5 Hz)). Proton H_g may be compared with a somewhat analogous proton in plumericin.[8] Structural moiety **2** is thus firmly estab-



1a, R = H_c; R′ = OH
b, R, R′ = O
c, R = H; R′ = OAc



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[1] This was a cooperative investigation sponsored by the Agricultural Research Service, USDA, Contract No. 12-14-100-8308(34), administered by the Crops Research Division, Beltsville, Md. X-Ray investigations were supported through a Biomedical Sciences Support Grant to Duke University.

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[6] Formula from high-resolution mass spectra.

[7] δ in parts per million from internal tetramethylsilane at 100 MHz in $CDCl_3$.

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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 ($J_{h,j}$ slightly < $J_{i,j}$ = 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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