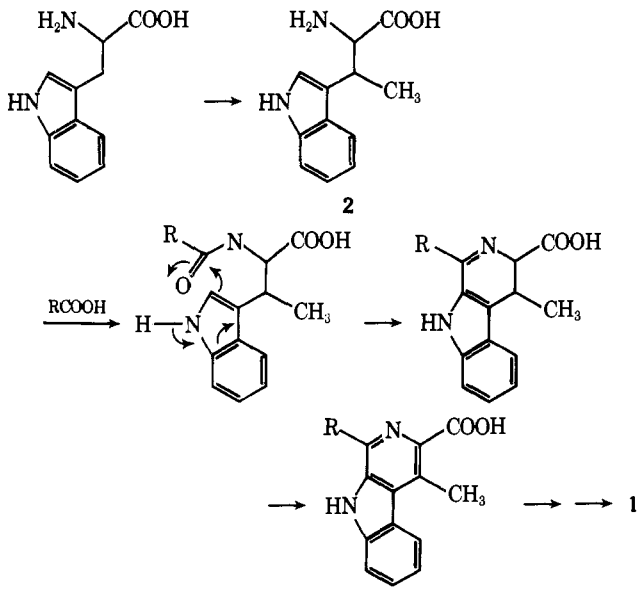
Scheme II



from the tryptophan feeding was isolated as barium carbonate, the complete lack of radioactivity in the *N*-methylbenzamide clearly locates the tryptophan label at C-3′. This gave us the opportunity to evaluate the somewhat tentative ^{13}C NMR assignments of the pyridine carbons.[7] [3-^{13}C]-DL-Tryptophan was synthesized,[16] and 82 mg (0.40 mmol, 90% enriched) were fed into eight of the large fermentation flasks. Workup afforded 76 mg of streptonigrin. Only the signal at 137.8 ppm of the ^{13}C NMR spectrum was enhanced.[17] Lown[7] had assigned this to C-4′ and the signal at 135.3 ppm to C-3′.

These results support the biosynthetic pathway shown in Scheme II. Tryptophan is methylated to β-methyltryptophan[15] (**2**) and condenses with a quinolinecarboxylic acid (or precursor thereof). Formation of the pyridine ring then results from the intramolecular attack on an amide carbonyl by the nucleophilic α position of the indole, followed by aromatization of the resulting dihydropyridine and cleavage[18] of the indole ring. This represents a new pathway for the formation of pyridine rings[19] and a new metabolism of tryptophan[20].

It is apparent from our labeling experiments that an intact tryptophan is not incorporated into the quinoline quinone A–B ring system of **1**. This rules out two of the three known pathways to quinoline rings.[21] We are currently investigating the origin of the quinoline quinone portion.

Acknowledgments. We wish to thank Mr. James Knittel for recording the ^{13}C NMR spectra. We are indebted to Dr. John DeZeeuw of Pfizer and Co., Inc., Groton, Conn., for strains of *S. flocculus*, to Dr. John Douros of Drug Research and Development, Chemotherapy, NCI, for a generous gift of streptonigrin, to Dr. David Cane of Brown University for a gift of [$^{13}CH_3$]-L-methionine, and to Dr. Richard Hutchinson of the University of Wisconsin for a gift of $^{13}CH_2O$. Drs. Robert Thomas and Ian Spenser are thanked for helpful discussions. This work was supported by a grant from the University of Connecticut Research Foundation to S.J.G.

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(17) Cleaner spectra can be obtained in Me_2SO-d_6 since this solvent permits more concentrated solutions and, unlike pyridine, does not give solvent signals which overlap with the solute spectrum. In Me_2SO-d_6 the signal at 136.2 ppm, which we now assign to C-3′, was enhanced eightfold relative to the signal at 180.3 ppm.
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The Intramolecular Hydrogen Bond in Malonaldehyde as Determined by X-Ray Photoelectron Spectroscopy

Sir:

Recently the intramolecularly H-bonded enol forms of certain 1,3 diketones (**1–3**, Scheme I) have been examined by theoretical[1] and experimental[2] techniques with the goal of deciding whether the enol forms are best represented as an asymmetric C_s structure (**a** or **c**) or a symmetric C_{2v} structure (**b**). We wish to report results concerning O_{1s} binding energies obtained from x-ray photoelectron spectroscopy for compounds **1–3** and for the nonenolized 3,3-dimethylacetylacetone (**4**) which shed light on this fundamental question.

It is expected that the symmetric C_{2v} form (**b**) should show a single ionization from its equivalent oxygens, while the asymmetric form (**a** or **c**) should give rise to two different ionizations which might be resolvable or contribute to a broadened signal. Table I shows the O_{1s} binding energies for malonaldehyde (**1**),[3] acetylacetone (**2**),[6a] hexafluoroacetylacetone (**3**),[6b] and 3,3-dimethylacetylacetone (**4**).[7]

The data for the nonenolizable **4** clearly indicate a single O_{1s} ionization (half-width = 1.77 eV). On the other hand **1**, which is entirely enolized in solution[3] and presumably so in the gas phase,[2a] shows an O_{1s} ionization which deconvolutes into a small peak[8] and two larger ones in a 1:1 area ratio. In the ab-

Table I. O_{1s} Binding Energies for 1,3-Dicarbonyls[a,b]

Compd	Peak[c]	Relative[d] area	Binding energy, eV	fwhm,[e] eV
Malonaldehyde (1)	1	0.16 ± 0.05	542.52	1.60
	2	0.96 ± 0.05	539.71	1.49
	3	1.0 ± 0.04	538.14	1.62
Acetylacetone (2)	1	0.10 ± 0.01	541.68	1.86
	2	1.43 ± 0.08	538.83	1.95
	3	1.00 ± 0.11	537.33	1.65
3,3-Dimethylacetyl-acetone (4)	1	1.0	538.08	1.77
Hexafluoroacetyl-acetone (3)	1	0.37 ± 0.03	543.06	2.24
	2	1.47 ± 0.17	540.48	1.75
	3	1.66 ± 0.16	539.03	1.91

[a] Each binding energy is the average of four determinations and is calibrated against a Ne auger line 804.56-eV kinetic energy (T. D. Thomas and R. W. Shaw, *J. Elect. Spectrosc.*, **5**, 1081 (1974)). [b] Spectral data were computer analyzed using an ELSPEC program previously described (R. S. Brown and D. A. Allison, *J. Am. Chem. Soc.*, in press). [c] See footnote 8. [d] Errors are from least-squares fit. [e] Full width at half maximum height.

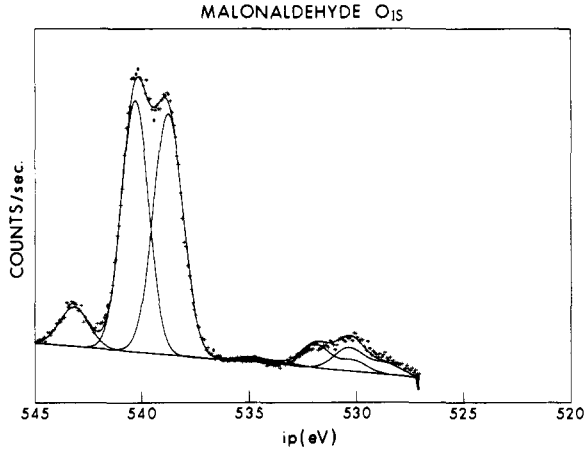


Figure 1. An unrestricted computor deconvolution of the O_{1s} ionization region of malonaldehyde. See footnote 8.

sence of a keto form, the spectrum cannot be consistent with ionization from a single symmetrical species. Since two equal intensity peaks are expected from the C_s structure (assuming equal ionization cross sections for the inequivalent oxygens) the observed spectrum is best interpreted as arising from enol forms **a** or **c**. Figure 1 shows unrestricted computor deconvolution of the data.[10]

This observation complements the interpretation of Rowe, Duerst, and Wilson[2a] who suggest, on the basis of microwave spectroscopy, that the molecule exists in asymmetric forms **a** and **c** with a low barrier to interconversion via form **b**.

In the case of **2**, the O_{1s} ionization region also shows two dominant peaks in an approximate 6:4 ratio; however, it seems certain from other data[2b,11] that the molecule is not completely enolized and thus ionization from a keto form and some enol form must be occurring simultaneously giving rise to the observed spectrum. Further analysis in terms of the dominant enol form is precluded at this time.

However, for **3**, which is apparently completely enolized in the gas phase,[2c] the deconvoluted spectrum shown in Figure 2 distinctly shows two dominant peaks[8] which within experimental error have a 1:1 ratio. This observation is only consistent with a single enolized species in which the two oxygens are inequivalent by virtue of an asymmetric H bond. This result contrasts the assumed symmetric location of the hydrogen derived from the electron diffraction study.[2c]

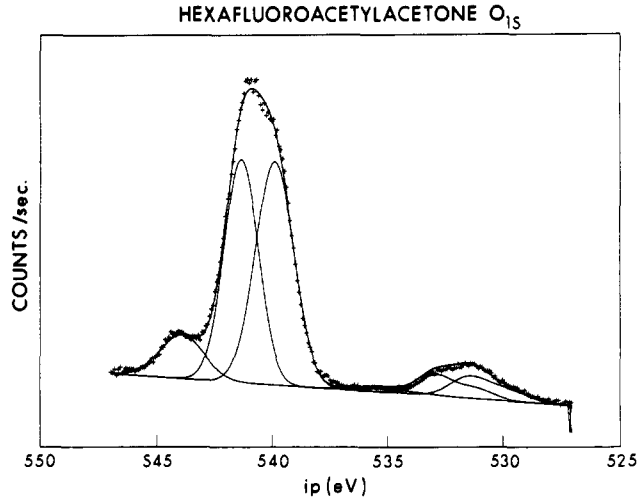
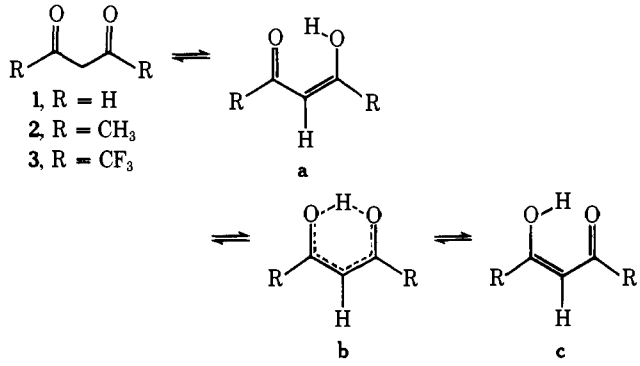


Figure 2. An unrestricted computor deconvolution of the O_{1s} ionization region of hexafluoroacetylacetone. See footnote 8.

Scheme I



Our findings for malonaldehyde can be compared with the calculations of Karlström et al.[1a] who predicted that the asymmetric enol form of this molecule should show two O_{1s} ionizations separated by roughly 1 eV.[12] The experimentally observed separation is 1.57 eV.

Acknowledgment. The author gratefully acknowledges the assistance of the National Research Council of Canada and the University of Alberta. He is also indebted to Dr. David Allison for invaluable assistance with technical and computing matters.

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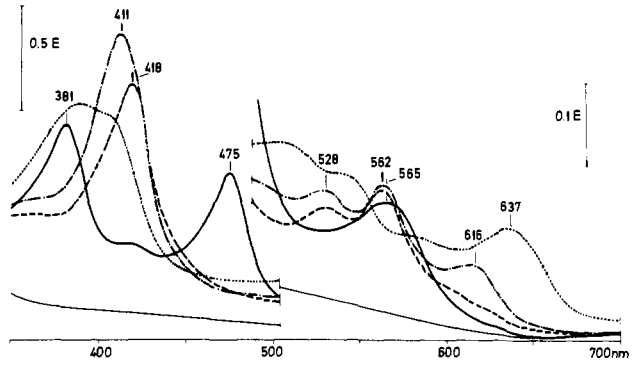

Figure 1. Electronic spectra of hemin-mercaptide complexes at 77 K. Chlorohemin (2 mM) in DMF (• • •) was titrated with mercaptide (0.1 M *n*-BuSH with 0.1 M $(CH_3)_4NOH$)[10] at −60 °C. The mercaptide/heme ratios were 1 (- • -),[8] 2 (- - -),[8] and 4.9 (—). The baseline (—) was recorded with DMF. The optical path length was $\sim 5 \times 10^{-3}$ cm.

Hyperporphyrin Spectra of Ferric Dimercaptide-Hemin Complexes. Models for Ferric Cytochrome P450-Thiol Complexes

Sir:

Cytochrome P450, catalyzing various monooxygenase reactions,[1] exhibits unusual spectroscopic properties, nearly all of which have been mimicked with heme complexes liganded with one mercaptide sulfur. Such model complexes have successfully been prepared for the ferric states[2,3] and for the ferrous carbonyl[4] and nitrosyl[5] complexes of cytochrome P450. Its prominant feature, the Soret band at 450 nm of the ferrous CO complex, has been identified by Hanson et al.[6] to be part of a "hyperporphyrin" spectrum. A common characteristic of a number of hyperporphyrin spectra are two intense "Soret bands", one in the 370-380-nm region and another in the 440-480-nm region. An interpretation was given using extended Hückel calculations and the very plausible assumption of mercaptide as a trans ligand. This mercaptide provided a lone-pair sulfur orbital, suitable for a charge-transfer transition to the porphyrin e_g (π^*), and the strong interaction of this transition with the porphyrin a_{1u} (π), a_{2u} (π) → e_g (π^*) transition could result in the intense UV and red-shifted Soret bands. This interpretation was given for ferrous heme mercaptide complexes, but our observation of Soret bands between 455 and 475 nm for complexes of ferric cytochrome P450 with organic thiols and phosphines[7] seemed to be a sign of hyperporphyrin spectra, also of ferric heme complexes. In this communication we present electronic and ESR spectra of ferric hemin-mercaptide models: hyperporphyrin spectra were found for dimercaptide-hemin (and mercaptide-phosphine-hemin) complexes which simulated the spectroscopic properties of the corresponding ferric cytochrome P450 complexes very closely.

For our model studies, hemin solutions were titrated with mercaptide below −60 °C. This temperature was sufficiently low to prevent the reduction of the hemin. The samples were frozen in liquid nitrogen, and electronic and ESR spectra were recorded from the same sample, using ESR quartz sample tubes with 2.8-mm i.d. with a glass insert to reduce the optical path length down to 0.05 mm. This procedure overcame the difficulty that ESR usually required a second, more concentrated sample compared to optical spectroscopy. The electronic spectra of such a titration are shown in Figure 1. At a mercaptide/heme ratio of >3, two Soret bands were observed at ~380 and 470 nm and a broad band at 565 nm, a spectrum very similar to that of cytochrome P450-thiol complexes.[8] Since both Soret peaks appear or disappear concomitantly always, we assigned one hyperporphyrin species to this spectrum. The corresponding ESR spectrum (Figure 2), with the determination of the spin concentration, clearly demonstrates the low spin ferric nature of this spectral species. Although the spectral data varied a little with the mercaptide and the solvent used (Table I), the hyperporphyrin type spectrum did not change.[9] The hyperporphyrin spectrum remained even at 30-fold excess of mercaptide or when an anaerobic procedure was used which maintained a concentration of O_2 of $<10^{-5}$ M corresponding to <1% of the concentration of hemin. The hyperporphyrin spectrum did not appear when a 10-fold excess of either mercaptane or $(CH_3)_4NOH$ was added to a solution with a mercaptide/heme ratio of 1. The addition of 1.5 equiv of imidazole to the hyperporphyrin species was sufficient to abolish the hyperporphyrin spectrum completely with concomitant shifts of the ESR values (Table I), suggesting ligand exchange of one mercaptide by imidazole.[10] From these findings we assign the hyperporphyrin spectrum to ferric heme with two axial mercaptide ligands.

The good simulation of the spectra of cytochrome P450-

Table I. Spectroscopic Data of Hemin-Mercaptide Complexes

Complex	Ligand/heme	Electronic spectrum max, nm	ESR spectrum (at 100 K), *g*
FePPIXDME + *n*-$C_4H_9S^-$ [a]	10	377, 475, 565	2.310, 2.227, 1.958
FePPIXDME + $C_6H_5CH_2S^-$ [a]	3.4	376, 470, 561	2.302, 2.228, 1.959
FePPIXDME + $C_6H_5S^-$ [a]	3.4	387, 473, 565	2.385, 2.262, 1.936
FePPIXDME + p-$NO_2C_5H_4S^-$ [a]	2.5	376, 455, 555, 611	2.405, 2.274, 1.925
FePPIXDME + $C_6H_5CH_2S^-$ + Im[a]	3.4, 1.5	428, 538, 568	2.363, 2.241, 1.937
FePPIXDME + *N*-MeIm[c]	2	409, 522, 558[d]	2.90, 2.29, 1.57
FePPIXDME + $C_6H_5CH_2S^-$ + Et_2PhP	3.4, 10	374, 458, 556 (585)	2.391, 2.264, 1.924
Cytochrome $P450_{CAM}$ + $C_6H_5CH_2SH$[b]		377, 465, 557[d]	(2.43), 2.37, 2.25, 1.94 (1.92)
Cytochrome $P450_{RLM}$ + *n*-$C_8H_{17}SH$[b]		378, 471, 552, 592[d,e]	(2.42, 2.26, 1.91)
Cytochrome $P450_{CAM}$ + Et_2PhP[b]		377, 454, 556[d]	2.50, 2.28, 1.88

[a] Solvent CH_2Cl_2, mixing temperature −78 °C, electronic spectra at 77 K. [b] Reference 7, CAM from *Pseudomonas putida*, RLM rat liver microsomes. [c] Reference 2b, no mercaptide complex. [d] Ambient temperature. [e] Difference spectrum.