Table I. Molecular Constants of the $^3B_1$ State (cm^{-1})

Term value (band origin)	Rotational constants	Centrifugal constants	Spin–rotation constants	Spin–spin constants
25765.79	A_{000} 2.3129	10^4D_N 0.0044	a_0 −0.005	α 0.115
	B_{000} 0.2970	10^4D_{NK} −0.080	a −0.008	β (0.03)
	C_{000} 0.2624	10^4D_K 2.84	b (0)	
		10^8H_K 7.9		

of ^{16}O nuclei *prove* that the electronic symmetry is $^3B_1$; and (iii) the electric-dipole-active spin function is $|b\rangle$; thus the transition gains its intensity by spin–orbit coupling of $^3B_1$ with a singlet state of B_2 orbital symmetry. The electronic symmetry and spin–orbit coupling mechanism are the same as those recently established for the isovalent molecule NO_2^-.[4,5] Some of the results described here are foreshadowed in the excellent work of Merer, who, however, was not successful in analyzing the spin splittings.[6]

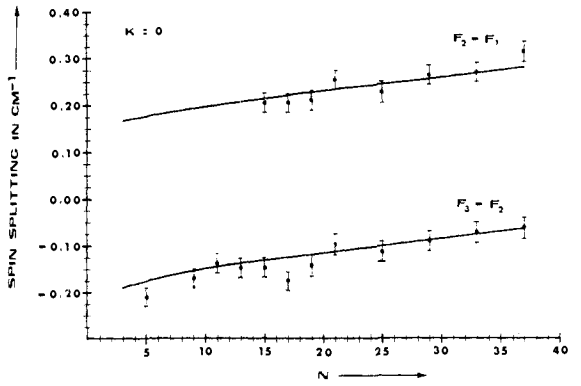

Figure 1. Spin splittings $F_2 - F_1$ and $F_3 - F_2$ for $K' = 0$. The vertical lines represent the standard deviation in the fit (see text).

In the triplet state, except when $N - K$ is small, the spin sublevels in order of decreasing energy are F_2 ($J = N$) > F_3 ($J = N - 1$) > F_1 ($J = N + 1$), the splittings being typically 0.1–0.2 cm^{-1}. Splittings observed for the $K' = 0$ manifold are shown in Figure 1, the curves being calculated by diagonalization of the complete energy matrices for the asymmetric rotor in a triplet state,[7] using values of the spin–spin and spin–rotation constants given in Table I. The sequence $F_2 > F_3 > F_1$ indicates a positive value for the dominant spin–spin constant α,[8] combined with relatively small constants for spin–rotation coupling. Zero-field splittings are not resolved in the triplet–singlet crystal spectrum of SO_2,[9] possibly by a narrow margin.

In the low K subbands, where the effects of asymmetry are greatest, the intense rotational branches are $^rS_3$, $^pS_3$, $^rQ_3$, $^rR_2$, $^pP_2$, $^pQ_1$, $^pO_1$, and $^rO_1$.[10] Except for small values of N, the $^rS_3$ and $^pS_3$ branches run together and generate (about 9 cm^{-1} to high frequency of the band origin) the prominent "spike" characteristic of the triplet–singlet bands of SO_2 as seen under low resolution: its mechanism of formation is then similar to that which produces the Q branch of ordinary C-type infrared transitions. The intensity distribution in the subbands is characteristic of singlet–triplet mixing induced by a spin function of a_2 symmetry, *i.e.*, the active function $|b\rangle$.[11,12] The fact that the branches all conform to the rule $\Delta K_{\text{prolate}} = \pm 1$ means that the triplet-state symmetry must be $^3A_1$ or $^3B_1$, but does not of itself distinguish between these possibilities. However, owing to the zero spin of ^{16}O nuclei, one-half the rotational levels are missing in each state of the transition: in the triplet state the missing levels have even values of τ and are compatible only with the assignments $^3B_1$ or $^3B_2$,[6,11,12] so that the observations jointly prove that the symmetry is $^3B_1$. This confirms the identification, current for many years, with the triplet state of the first excited electronic configuration, $\ldots(6a_1)^1(2b_1)^1$; $^{1,3}B_1$.

As the spin greatly increases the core requirement for least-squares procedures, the constants in Table I are fitted to the frequencies of about 250 selected transitions only (standard deviation <0.02 cm^{-1}) and are considered reliable to the extent indicated. The r_0 structure calculated without regard to effects of vibrational amplitude is

$$r(SO) = 1.493 \text{ Å} \qquad \angle OSO = 126.2°$$

with probable error of about 1 part in 10^3. The corresponding ground-state constants are 1.432 Å and 119.5°;[13] thus the bond distance and angle both increase in the transition. The identity of the $^1B_2$ state which shares in the large matrix element for spin–orbit coupling is uncertain, though it may occur in the 3000-Å band system(s).[14] It is tempting to consider that this coupling is responsible also for the strong magnetic rotation spectrum known in that region.[15]

(4) R. M. Hochstrasser and A. P. Marchetti, *J. Chem. Phys.*, 50, 1727 (1969).
(5) W. C. Allen and R. N. Dixon, *Trans. Faraday Soc.*, 65, 1168 (1969).
(6) A. J. Merer, *Discuss. Faraday Soc.*, 35, 127 (1963).
(7) W. T. Raynes, *J. Chem. Phys.*, 41, 3020 (1964).
(8) α and β are related to the esr zero-field splitting parameters D and E: $\alpha = D/3$ and $\beta = E$.
(9) R. M. Hochstrasser and A. P. Marchetti, private communication.
(10) The numerical subscript indexes the triplet-state spin component.
(11) J. T. Hougen, *Can. J. Phys.*, 42, 433 (1964).
(12) C. di Lauro, *J. Mol. Spectrosc.*, in press.
(13) G. Herzberg, "Electronic Spectra of Polyatomic Molecules," Van Nostrand, Princeton, N. J., 1966, p 605.
(14) R. S. Milliken, *Can. J. Chem.*, 36, 10 (1958).
(15) P. Kusch and F. W. Loomis, *Phys. Rev.*, 55, 850 (1939).

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Addition of Hydrogen Atoms to Glutathione Disulfide in Aqueous Solution

Sir:

The inactivation of enzymes by hydrogen atoms in aqueous solution has been successfully correlated with the selective attack of H atoms at the disulfide bridges

in addition to the attack on tyrosine and/or tryptophan.[1] The rather high reaction rate of H atoms with the disulfide group in simpler molecules such as cystine ($k = 5 \times 10^9\ M^{-1}\ \text{sec}^{-1}$)[2,3] raises the question of the mechanism of the H-atom attack on the -S-S- linkage. We present here evidence that such attack does not lead to the initial scission of the S-S bond but rather the formation of the H-atom-adduct radical, -Ṡ-SH-.

Hydrogen atoms were produced by pulse radiolysis and the transients observed by kinetic absorption spectroscopy.[4] A 30-nsec pulse of 2.4-MeV electrons caused the radiolytic decomposition of water and the formation of primary radicals: $H_2O \rightsquigarrow 3.0e_{aq}^- + 3.0OH + 0.6H$. In acidic solutions (pH <2), e_{aq}^-'s are efficiently converted into H atoms: $e_{aq}^- + H_3O^+ \rightarrow H$ ($k = 2 \times 10^{10}\ M^{-1}\ \text{sec}^{-1}$).[3] The OH radicals can be removed, if desired, through their reaction with *tert*-butyl alcohol ($k = 5 \times 10^8\ M^{-1}\ \text{sec}^{-1}$)[3] with the formation of weakly absorbing transients (λ_{max} 225 nm; ϵ_{225} 900 $M^{-1}\ \text{cm}^{-1}$),[5] which appear to be relatively unreactive toward many solutes; the rate of H-atom reaction with *tert*-butyl alcohol has been shown to be very low ($k \leqslant 10^5\ M^{-1}\ \text{sec}^{-1}$).[6] All these features suggest that *tert*-butyl alcohol is one of the most convenient scavengers for the elimination of OH radicals in the study of H-atom reactions. Aqueous acidic solutions of glutathione[7] (γ-Glu-Cys-Gly; represented as RSH) and glutathione disulfide[7] (RSSR) were pulsed[5] in the presence and absence of *tert*-butyl alcohol.

The reaction of H atoms with RSH produces a weak transient absorption spectrum (Figure 1a, circles, corrected for the contribution of the *tert*-butyl alcohol radicals).[5] Since the reactivity of the -SH group with H atoms is $>10^9\ M^{-1}\ \text{sec}^{-1}$ and the reactivity of any particular C-H group in the same compound is $\leqslant 10^7\ M^{-1}\ \text{sec}^{-1}$,[3] it can safely be assumed that the attack of H atoms takes place predominantly at the -SH position. These kinetic considerations are in agreement with the results of the γ radiolysis of glutathione (RSH),[8] where the main products at low pH were found to be H_2 and RSSR (the latter presumably formed from the combination of RS· radicals) with RH and H_2S as minor products (<25% of the radical yield). On the basis of these facts, the spectrum in Figure 1a (circles) is attributed to the radicals resulting from

$$H + RSH \longrightarrow RS\cdot + H_2 \quad (1)$$

$$\longrightarrow R\cdot + H_2S \quad (2)$$

with reactions 1 and 2 occurring at a greater than 3:1 ratio as indicated by the product analysis.[8] In the absence of *tert*-butyl alcohol, the transient spectrum obtained (Figure 1a, squares) from the reaction of H atoms and OH radicals is not different from that resulting from H-atom reaction alone. The approximate doubling of the yield of the transient when the OH reaction with RSH is also involved (in the absence of *tert*-butyl alcohol) is consistent with the relative yields of H and OH from the decomposition of water. These results suggest

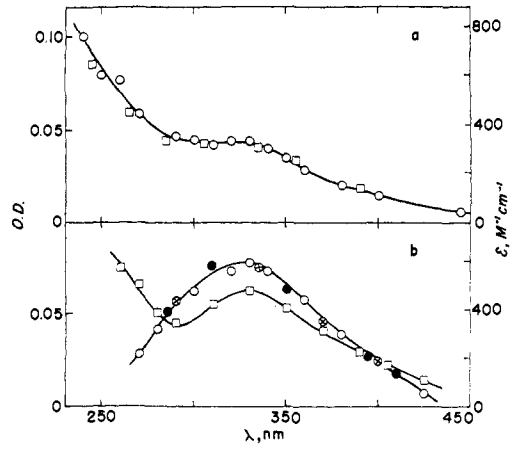

Figure 1. Absorption spectra from the pulse radiolysis of aqueous solutions of glutathione and glutathione disulfide. (a) 2 m*M* glutathione (RSH) at pH 1.0: ○, in the presence of 1 *M tert*-butyl alcohol (corrected spectrum), dose/pulse ~19 krads; □, in the absence of *tert*-butyl alcohol, dose/pulse ~8 krads. (b) 2 m*M* glutathione disulfide (RSSR) in the presence of *tert*-butyl alcohol (corrected spectra), dose/pulse ~19 krads: ●, pH 1.0; ○, pH 1.6; ⊗, pH 3.7; □, in the absence of *tert*-butyl alcohol, pH 1.0, dose/pulse ~8 krads.

$$OH + RSH \longrightarrow RS\cdot + H_2O \quad (3)$$

and that the rate of combination of the *tert*-butyl alcohol radical is much higher under our experimental conditions than is the rate of the repair reaction[9]

$$\cdot CH_2(CH_3)_2COH + RSH \longrightarrow (CH_3)_3COH + RS\cdot \quad (4)$$

The reaction of H atoms with RSSR at low pH values results in a transient absorption spectrum with λ_{max} 330 nm (Figure 1b, filled and open circles), which is significantly different from that shown in Figure 1a. On this basis we can exclude consideration of the scission reaction to form RS· and RSH. Rather, we wish to suggest that H atoms add at the disulfide group. This suggestion is considerably strengthened by the result that the transient absorption spectrum remains unchanged at pH 3.7 (Figure 1b, circles with ×) where the initial reaction is that of e_{aq}^- with RSSR ($k > 4 \times 10^9\ M^{-1}\ \text{sec}^{-1}$)[3]. The species thus produced is $(RSSR)^-$ which is rapidly protonated at times shorter than our experimental resolution (~1 μsec). In the presence of 25 m*M* N_2O at pH 3.7 this RṠSHR absorption is almost completely suppressed, since under these conditions the hydrated electrons react predominantly with N_2O ($k = 5.6 \times 10^9\ M^{-1}\ \text{sec}^{-1}$)[3] rather than with RSSR.

While the H-atom adduct to the disulfide group, RṠSHR, has not been reported previously, the conjugated anionic form, $(RSSR)^-$, has been observed in the pulse radiolysis of various aqueous systems such as hydrogen sulfide,[10] simple mercaptans,[11] cysteamine-

(1) L. K. Mee, G. Navon, and G. Stein, *Biochem. Biophys. Acta*, **104**, 151 (1965); R. Shapira and G. Stein, *Science*, **162**, 1489 (1968).
(2) G. Navon and G. Stein, *Israel J. Chem.*, **2**, 151 (1964).
(3) M. Anbar and P. Neta, *Int. J. Appl. Radiat. Isotopes*, **18**, 493 (1967).
(4) For example, M. S. Matheson and L. M. Dorfman, "Pulse Radiolysis," MIT Press, Cambridge, Mass., 1969.
(5) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, **73**, 3794 (1969).
(6) G. Scholes and M. Simic, *ibid.*, **68**, 1738 (1964).
(7) Compounds obtained from Calbiochem (Grade A).
(8) M. Lal, D. A. Armstrong, and M. Wieser, *Radiation Res.*, **37**, 246 1969).
(9) G. E. Adams, G. S. McNaughton, and B. D. Michael, *Trans. Faraday Soc.*, **64**, 902 (1968).
(10) W. Karmann, G. Meissner, and A. Henglein, *Z. Naturforsch. B*, **22**, 273 (1967).
(11) W. Karmann, A. Granzow, G. Meissner, and A. Henglein, *Int. J. Radiat. Phys. Chem.*, **1**, 395 (1968).

cystamine,[12] cysteine–cystine,[13] and lysozyme.[14] These radical anions exhibit λ_{max} ~410 nm with $\epsilon \sim 10^4$ M^{-1} cm^{-1} and are eliminated below pH 4 presumably due to protonation of the anionic form of the substrate or of the radical anion itself. Indeed, we found that the electron adduct to glutathione disulfide at pH 9 showed λ_{max} 420 nm and $\epsilon_{420} > 5 \times 10^3$ M^{-1} cm^{-1}.

The following scheme summarizes our results for glutathione disulfide.

$$\begin{array}{ccc} e_{aq}^- + RSSR & \longrightarrow & (RSSR)^- \\ \downarrow H^+ & & -H^+ \uparrow\downarrow H^+ \\ H + RSSR & \longrightarrow & R\dot{S}SHR \end{array} \qquad (5)$$

The H-atom adduct and the protonated form of the electron adduct are apparently identical, with λ_{max} 330 nm and ϵ_{330} 600 M^{-1} cm^{-1} for these nonresonance structures, as compared with the longer wavelength and higher ϵ values for the $(RSSR)^-$ form. We find that this latter species undergoes first-order decay at pH 9–12

$$(RSSR)^- \longrightarrow RS\cdot + RS^- \qquad (6)$$

with $k_6 = 2.5 \times 10^5$ sec^{-1} (pH independent). The protonated form exhibits a second-order decay in the presence of *tert*-butyl alcohol with $2k = 2.4 \times 10^9$ M^{-1} sec^{-1}. Unfortunately, any contribution to the decay rate due to the reaction of *tert*-butyl alcohol radicals with RSSHR cannot be excluded at the present time. Note that when both OH radicals and H atoms react with $R\dot{S}SR$, the resulting spectrum (Figure 1b, squares) exhibits the H-atom transient absorption at 330 nm as well as an increased absorption below 290 nm which we attribute to the OH attack.

Observations similar to those reported here have been made for the reaction of H atoms with cysteine–cystine, and the detailed results will be published in the near future.[15]

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(12) G. E. Adams, G. S. McNaughton, and B. D. Michael in "The Chemistry of Ionization and Excitation," G. R. A. Johnson and G. Scholes, Ed., Taylor and Francis, London, 1967, p 281.
(13) G. E. Adams, *Curr. Top. Radiat. Res.*, **3**, 35 (1967).
(14) G. E. Adams, R. L. Willson, J. E. Aldrich, and R. B. Cundall, *Int. J. Radiat. Biol.*, **16**, 333 (1969).
(15) E. Hayon, M. Z. Hoffman, and M. Simic, manuscript in preparation.
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Monoterpene Biosynthesis. III. Occurrence and Biosynthesis of Loganic Acid in Indole Alkaloid Synthesizing Plants

Sir:

Within the dicotyledonous angiosperms, the iridoid glucosides have been found in a broad diversity of structural types from which a general biogenetic pathway has not yet emerged.[1] It has been recognized, however, that methyl cyclopentano monoterpene glucosides which are hydroxylated at C-7 serve as intermediates in the biosynthesis of both secoiridoids[2] and indole alkaloids[3] of higher plants of the *Gentianaceae*, *Loganiaceae*, and *Apocynaceae* families. Hence Battersby observed that of three iridoids tested, monotropeine, genipin, and loganin, the latter alone acted as a precursor of the nontryptamine moiety of the indole alkaloids.[3] In retrospect this is also consistent with the congener relationship of loganin and strychnine in *Strychnos nux vomica* fruit.[4] We now have evidence for the occurrence and biosynthesis of the related iridoid, loganic acid, in the indole alkaloid synthesizing plants *Vinca rosea* and *Strychnos nux vomica*.

Seeds of both *Vinca rosea* and *Strychnos nux vomica* were found to contain relatively large quantities of loganic acid, accounting for 1% of their total weight. This concentration is considerably higher than the 0.05% distribution on a fresh weight basis observed for loganic acid in mature *Vinca* plants. Derivatives of the loganic acid from both plants possessed superimposable spectra with those of loganic acid isolated from *Swertia caroliniensis*.[6] This included optical rotation, uv, ir, nmr, and mass spectrometry. Melting points of admixtures of methylated loganic acid or loganin pentaacetate with authentic samples (provided by J. Wolinsky of Purdue University, Lafayette, Ind.) showed no depression.

Small amounts of loganin could also be isolated from *Strychnos* seeds whereas its presence in *Vinca* was ascertained[3] by isotope dilution studies (0.01% incorporation of mevalonate-2-^{14}C).

Feeding experiments were carried out in essentially three different ways. Germinating *Vinca* seeds were allowed to absorb water containing mevalonate-2-^{14}C (experiment 2, Table I) or 2-month old seedlings were fed mevalonate hydroponically (0.52% incorporation). With mature *Vinca* plants the cotton wick technique[6] was utilized (experiments 1 and 3, Table I).

Upon addition of 15 mg of carrier, loganic acid was isolated by the ion exchange method as previously described.[6] The crude acid was methylated and acetylated. The resulting loganin pentaacetate was recrystallized to constant activity and saponified back to loganin. The loganin was recrystallized to constant radioactivity and the data reveal relatively high rates of incorporation.

Emulsin hydrolysis[7] of loganin provides the aglucone, loganetin, and glucose. Determination of their respective specific activities established exclusive incorporation of mevalonate-2-^{14}C into the isoprenoid moiety (experiment 1, Table I). Decarboxylation studies

(1) J. M. Bobbitt, and K.-P. Segebarth, in "Cyclopentanoid Terpene Derivatives," W. I. Taylor and A. R. Battersby, Ed., Marcel Dekker, New York, N. Y., 1969, pp 1–145.
(2) C. J. Coscia, L. Botta, and R. Guarnaccia, *Arch. Biochem. Biophys.*, **136**, 498 (1970).
(3) A. R. Battersby in "Natural Substances Formed Biologically from Mevalonic Acid," T. W. Goodwin, Ed., Academic Press, London, 1970, pp 157–168.
(4) In 1951 Jaminet[5] discovered a compound in *Strychnos nux vomica* which he identified as loganic acid on the basis of its chromatographic mobility, uv spectra, and appearance upon saponification of loganin.
(5) Fr. Jaminet, *Lejeunia*, **15**, 23 (1951); *J. Pharm. Belg.*, **35**, 339 (1953).
(6) C. J. Coscia, R. Guarnaccia, and L. Botta, *Biochemistry*, **8**, 5036 (1969).
(7) G. Büchi and R. E. Manning, *Tetrahedron*, **18**, 1049 (1962).