Table I. Effect of EDTA Chelation of the Apparent Uncatalyzed Oxidation of Aqueous Sulfite Solutions

solution A[a]		solution B[c]	
EDTA, M	rate, 10^{-5} M/min	EDTA, M	rate, 10^{-5} M/min
0.0	149	0.0	(14 700)[d]
1.0×10^{-8}	65	5.0×10^{-7}	23
5.0×10^{-8}	35	1.0×10^{-6}	3.3
1.0×10^{-7}	26	5.0×10^{-6}	1.5
1.0×10^{-6}	0.2	1.0×10^{-4}	0.2

[a] 0.003 M Na_2SO_3 (6×10^{-8} M Cu impurity [b] by atomic absorption analysis. [b] Reference 9. [c] 0.02 M Na_2SO_3 ($2.3 \times$ Cu impurity[b] by atomic absorption analysis. [d] Calculated from the Cu analysis plus the data of Mishra and Srivastava.[9]

sulfite oxidation since it is most effective in the high pH range, where it acts as a sexadentate ligand, occupying all the coordination sites of the complexed metal ion. 1,10-Phenanthroline was chosen in the low pH sulfur dioxide reaction since, unlike EDTA, its chelating effectiveness is not significantly reduced by acidity. It is also important to note that both chelating agents used are not capable of acting as an oxidation inhibitor in the classic sense of terminating free-radical chains. The effects of EDTA and 1,10-phenanthroline were investigated at 25 °C in a quartz Morton reactor modified to facilitate oxygen mass transfer and allow for rapid sample withdrawal. The kinetics were established by monitoring the sulfur(IV) concentration iodometrically as a function of time. The high pH (9.2-9.4) sulfite solutions were prepared using sodium sulfite salt while the low pH (1.65-1.95) sulfur dioxide solutions were prepared by sparging SO_2 gas into solution.

By using two separate sources of highly purified water, denoted as A and B, high pH kinetic studies were performed on two sets of sulfite solutions of significantly differing levels of trace-metal impurities. Atomic absorption analysis (Jarrell Ash Model 810 spectrophotometer) detected Cu and Fe impurities of 6×10^{-8} and 8×10^{-9} M, respectively, in water source A with corresponding impurity levels of 2.3×10^{-7} and 5×10^{-7} M in source B. Co and Mn, the other potentially active catalysts, could not be detected within the limits of analysis ($\sim 1 \times 10^{-8}$ M) in either water supply. Kinetic studies of the low pH reaction were all performed using deionized water from source B.

The effect of EDTA concentration on the rate of the apparent uncatalyzed sulfite oxidation is shown in Table I. Note that for both solutions studied very marked reductions in the rate were observed at relatively low EDTA concentrations, with the rate reduced over four orders of magnitude in solution B at an EDTA concentration of 1.0×10^{-4} M. Also note that the reaction in solution B, in the absence of EDTA, was mass-transfer limited. The rate quoted in Table I is quite approximate and has been estimated from the known Cu impurity level and extrapolation of the kinetic data of Mishra and Srivastava.[9]

The effect of 1,10-phenanthroline on the apparent uncatalyzed oxidation rate of low pH sulfur dioxide solutions is shown in Table II. Note that, although the effects are much less marked than those observed in the high pH reaction, the apparent uncatalyzed oxidation rate is substantially reduced by the addition of 1,10-phenanthroline which complexes the iron impurities in solution.[10]

These results clearly suggest that the oxidations of both high pH sulfite and low pH sulfur dioxide solutions, previously accepted as uncatalyzed, are in fact due primarily to trace-metal impurities acting as catalysts. Note, however, that measurable reaction rates were still obtained in the presence of excess chelating agent in both the high and low pH solutions, suggesting that the reaction may also proceed by some parallel

Table II. Effect of 1,10-Phenanthroline on the Apparent Uncatalyzed Oxidation of Aqueous Sulfur Dioxide Solutions

1,10-phenanthroline, 10^{-5} M	reaction rate, 10^{-7} M/min $[S(IV)]_0$, 0.050 M[a]	$[S(IV)]_0$, 0.015 M[a]
0.0	68.1	10.5
0.05		10.4
1.0	35.4	
5.0		4.98
10.0	10.7	
100.0	3.45	1.98

[a] 5×10^{-7} M Fe impurity[b] by atomic absorption analysis. [b] Reference 10.

thermal or photoinitiated reaction, but at a greatly reduced rate. It should also be noted that, although this is the first such evidence reported on the oxidation of sulfur(IV) solutions, other studies[11,12] have revealed trace-metal catalysis in certain previously accepted uncatalyzed hydrocarbon autoxidations.

The conclusions drawn from the high pH studies were further supported by a study of the comparative efficiencies of various aromatic free-radical inhibitors (phenol, hydroquinone, resorcinol, phloroglucinol, pyrogallol, and pyrocatechol) on both the Cu-catalyzed and apparent uncatalyzed reactions. Pyrocatechol, which is capable of chelating transition metal ions, was found to have a markedly greater inhibiting influence than the other inhibitors which have little or no complexing abilities.

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References and Notes

(1) S. L. Bigelow, *Z. Phys. Chem.*, **26**, 493 (1898).
(2) E. C. Fuller and R. H. Crist, *J. Am. Chem. Soc.*, **63**, 1644 (1941).
(3) D. Winkelman, *Z. Elektrochem.*, **59**, 891 (1955).
(4) E. Abel, *Monatsh. Chem.*, **82**, 815 (1951).
(5) L. C. Schroeter, *J. Pharm. Sci.*, **52** (6), 559 (1963).
(6) M. C. Rand and S. B. Gale, "Principles and Applications of Water Chemistry", S. D. Faust and J. V. Hunter, Ed., Wiley, New York, N.Y., 1967, pp 380-404.
(7) S. Beilke, D. Lamb, and J. Müller, *Atmos. Environ.*, **9**, 1083 (1975).
(8) S. Yagi and H. Inoue, *Chem. Eng. Sci.*, **17**, 411 (1962).
(9) G. C. Mishra and R. D. Srivastava, *Chem. Eng. Sci.*, **31**, 969 (1976).
(10) A comparative study of the catalytic effectiveness of various transition metal ions has indicated that in the high pH sulfite reaction Cu and Co are the most active. In the low pH sulfur dioxide oxidation Mn and Fe are very effective catalysts, while Co and Cu are quite inactive.
(11) N. Uri, *Chem Rev.*, **50**, 375 (1952).
(12) N. Uri, "Autoxidation and Antioxidants", Vol. I, W. O. Lundberg, Ed., Wiley, New York N.Y., 1961, Chapter 2.

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Role of Anhydrovinblastine in the Biosynthesis of the Antitumor Dimeric Indole Alkaloids

Sir:

For the past decade numerous attempts have been made in this laboratory[1] to establish the identity of those monomeric indole alkaloids which serve as the building blocks for the antitumor alkaloids of *Catharanthus roseus*, exemplified by vinblastine (VLB, **1**), vincristine (VCR, **2**), leurosine (**3**), and leurosidine (**4**).[2] Thus, while vindoline (**5**) appears to serve as a satisfactory, intact precursor for the *Aspidosperma* segment of **1** (0.05% incorporation[1]), the derivation of the "iboga-like"

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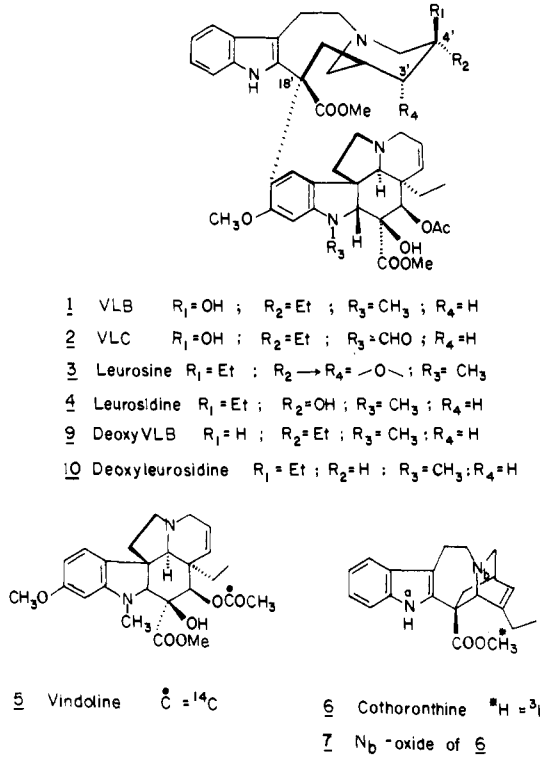
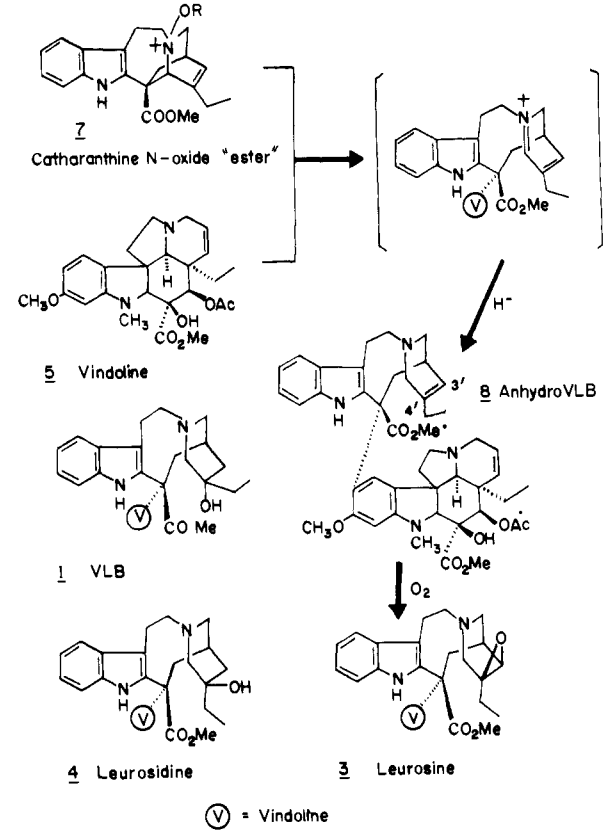
Table I. Incorporation of Vindoline **5** and Catharanthine **6** into Dimeric Alkaloids in *C. roseus* Plants

expt	precursor	feeding time, days	alkaloid isolated (% incorpn) anhydro-VLB (**8**)	VLB (**1**)	leurosine (**3**)
1	[Ar^{3}H]-**6**[a]	7	0.037	0.005	0.072
2	[-CO$_2$C^{3}H$_3$]-**6**[b]	6	0.031	0.001	0.055
3	[-CO$_2$C^{3}H$_3$]-**6**[c]	9	0.47		
4	[*acetyl*-^{14}C]-**5**[d]	7	0.04	0.005	0.13
5	[*acetyl*-^{14}C]-**5** + **6**[e]	6[f]	2.63	~0.005	0.50

[a] Prepared by treatment of catharanthine hydrochloride (30 mg) in ^3H_2O (0.1 mL, 100 mCi)/H_2O (0.09 mL) with $(CF_3CO)_2O$ (0.93 mL) (5 °C, 5 h); 14 mg (6.6 × 10^7 dpm/mg) were fed to 4 shoots of *C. roseus* followed by isolation and dilution with **3**, **1**, and **8** and crystallization to constant activity. [b] Prepared by hydrolysis of catharanthine (KOH-EtOH, reflux, 8 h) and reesterification (DMF/T_2O/CH_2N_2/Et_2O); 13.6 mg (6.99 × 10^7 dpm/mg) were administered as described in footnote *a*. [c] As in footnote *b* but longer feeding time. [d] Vindoline (100 mg) hydrolyzed with concentrated HCl (3 mL) (5 min, reflux) and the purified deacetyl compound (21 mg) reacetylated with [1-^{14}C]-Ac_2O (250 μCi) in dry pyridine; 6.5 mg (2.13 × 10^7 dpm/mg) were administered as in footnote *a*. [e] As in footnote *d* using 11.8 mg (5.71 × 10^7 dpm/mg) with the addition of catharanthine (11 mg). [f] Total workup time, 3 h.





moiety of these structures has not been secured. A recent report[3] summarizing our results[1] and those of the Wisconsin group suggests that catharanthine (**6**), although poorly incorporated (~10^{-3}%) into **1**, still remains a favorite contender for this role, a position which it has occupied since the recognition of its unique stereochemistry, for it is the only natural monomeric iboga alkaloid bearing the same chirality as the "iboga" segment of VLB (**1**).[4]

In view of the clinical importance of compounds **1**–**4**,[5] their very low abundance in *C. roseus*[2] has become a matter of concern. The experiments described in this communication suggest why VLB and its congeners may occur in trace amounts and pose the question: "How many of the biologically active dimeric alkaloids (**1**–**4**) are artefacts?" [6]

During the course of the feeding experiments listed in Table I, TLC analysis of *blank* incubations of vindoline and catharanthine[7] at pH 6–8 indicated (by autoradiography) that trace quantities (0.001%) of dimeric substances were being formed in the absence of the plant enzymes. This further suggested that, perhaps, via *N*-oxide formation (**6** → **7**), followed by condensation-reduction, some anhydro-VLB (**8**) was being produced,[8] although the presence of **8** had not been demonstrated in *C. roseus*. We considered that the known instability of **8** in solution, in particular its facile conversion to leurosine (**3**) and catharine,[6] may have precluded attempts to detect this compound in plant extracts. We have now verified the latter concept by feeding both [*acetyl*-^{14}C]vindoline (**5**) and [OC^{3}H$_3$]catharanthine (**6**) to 6-week-old differentiated *C. roseus* plants for 6 days and, by modifying the established methods for extraction and purification of the alkaloidal material,[9] show that both [*acetyl*-^{14}C]- and [*carbomethoxy*-^{3}H]-labeled anhydro-VLB (**8**) can be isolated with radiochemical incorporation of up to 2.63% (Table I). Thus anhydro-VLB is a natural product.[10]

The [^{14}C] label was located in **8** after crystallization to constant activity and hydrolysis[8] to deacetylanhydro-VLB (**8**, OAc = OH) which after extensive purification was devoid of radioactivity. The efficiency of incorporation of label into anhydro-VLB was found to depend on (a) the speed of workup procedure and (b) the presence of adequate pool sizes of both catharanthine and vindoline, presumably at the site of biosynthesis. Thus, when the total elapsed workup time was reduced from 10 to 3 h, the incorporation of vindoline, 11.8 mg

(5.7×10^7 dpm), rose from 0.04 to 2.63% in the presence of unlabeled catharanthine (11 mg). These conditions have not been fully optimized but the incorporations are almost three orders of magnitude greater than those of **5** and **6** into VLB, which remain unchanged[1,3] ($\sim 10^{-3}$%) regardless of workup time and conditions.

On the other hand, the incorporation of **5** and **6** into the epoxide, leurosine (**3**), although positive (0.1–0.5%) must be regarded with caution in view of the inherent in vitro chemistry of the anhydro-VLB (**8**) → leurosine (**3**) conversion reported by Potier.[6] Since the epoxide of leurosine corresponds to the 4′-hydroxyl group of leurosidine (**4**) rather than that of VLB (**1**), the biosynthesis of the latter may proceed either by stereospecific hydration of the 3′,4′ double bond of **8** or by reduction to give the 4′ epimers of deoxy-VLB (**9, 10**) which are subsequently hydroxylated to **1** and **4,** respectively.

The availability of anhydro-VLB (**8**) both biosynthetically and by partial synthesis[8] would gain further significance if transformation to VLB could be effected. To this end administration of [*acetyl*-^{14}C]-**8** to *C. roseus* plants was examined and found to yield leurosine (**3,** 2.9% incorporation) almost exclusively. The corresponding blank (1.16%) indicates that some epoxide formation is enzyme catalyzed in a 2-day feeding experiment but also reveals that VLB (**1**) and leurosidine (**4**) are not formed under these feeding conditions owing to (a) the instability of the anhydro-VLB and/or (b) compartmentalization of the VLB synthesizing enzymes.

With the realization that the anhydro dimer **8** is formed in quite high yield in the in vivo condensation of vindoline and catharanthine but can suffer extensive in vitro degradation (e.g., to leurosine), the stage is now set for a rational experimental approach to the bioconversion of **8** to the deoxy- (**9, 10**) and VLB-leurosidine (**1, 4**) series under controlled conditions. The fascinating question remains, whether based on the isolation of **8** in *C. roseus,* the biologically active compounds **1–4** are *all* in vitro artefacts produced from the enzymically formed anhydro-VLB.

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References and Notes

(1) Summarized by A. I. Scott in "Recent Advances in Phytochemistry", V. C. Runeckles, Ed., Plenum Press, New York, N.Y., 1975, Chapter 9.
(2) D. J. Abraham, "The Catharanthus Alkaloids", W. I. Taylor and N. R. Farnsworth, Ed., Marcel Dekker, New York, N.Y. 1975, Chapter 4.
(3) S. B. Hassam and C. R. Hutchinson, *Tetrahedron Lett.*, 1681 (1978).
(4) J. P. Kutney, D. E. Gregonis, R. Imhof, I. Itoh, E. Jahngen, A. I. Scott, and W. K. Chan, *J. Am. Chem. Soc.*, **97,** 5013 (1975).
(5) W. A. Creasey in ref 2, Chapter 7; R. C. DeConti and W. A. Creasey in ref 2, Chapter 8.
(6) N. Langlois and P. Potier, *J. Chem. Soc., Chem. Commun.*, 102 (1978), have raised a similar question as a consequence of the in vitro formation of leurosine (**3**) from anhydro-VLB (**8**) in chloroform solution.
(7) W. P. Watson, unpublished experiments, 1975.
(8) P. Potier, N. Langlois, F. Gueritte, and Y. Langlois, *J. Am. Chem. Soc.*, **98,** 7017 (1976), and references cited.
(9) G. H. Svoboda, N. Neuss, and M. Gorman, *J. Am. Pharm. Ass., Sci. Ed.*, **68,** 659 (1959); G. H. Svoboda, *Lloydia*, **24,** 173 (1961); G. H. Svoboda and D. A. Blake in ref 2, Chapter 2.
(10) The term "natural product" herein is defined as any compound found in nature (with particular reference to secondary metabolites) whether formed by enzymatic catalysis or spontaneous reaction as favored by the in vivo environment. It is becoming clear that many "natural products" are formed *nonenzymatically* as the result of nonspecific reactions involving redox agents, radicals, localized pH variation, and so on. In other words, the subsequent reactivity of unstable enzyme-generated intermediates (e.g., **8**) in secondary metabolism may explain the origin of a host of structures whose formation is either entirely chemical in origin (see ref **6**) or nonspecific in terms of subsequent enzymatic transformation(s).

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Inverted Redox Catalysis: Catalysis of Substitution on Ruthenium(II) by an Extraordinarily Labile Ruthenium(III) Metal Center

Sir:

Our attempts to study the kinetics of the substitution reactions of ruthenium(II) complex, $Ru(edta)(H_2O)^{2-}$

$$Ru(edta)(H_2O)^{2-} + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ru(edta)L^{2-} + H_2O \quad (1)$$

failed repeatedly. When slightly acidic solutions (typically 10^{-4} M) of the pentadentate EDTA complex of ruthenium(III), $Ru(edta)(H_2O)^-$ (from $Ru(edtaH)(H_2O)\cdot 4H_2O$)[1,2] were reduced over amalgamated zinc or Pt/H_2 and then mixed with deaerated ligand ($\geq 10^{-3}$ M isonicotinamide or pyrazine), the formation of the orange $Ru(edta)L^{2-}$ product followed zero-order kinetics (plots of absorbance vs. time were linear). With $Ru(edta)H_2O^{2-}$ in excess (2×10^{-4} to 2×10^{-3} M, [isonicotinamide] = 2×10^{-5} M), similar results were obtained. However, long reduction periods and extreme care in the exclusion of dioxygen gave slower, mixed first-order/zero-order decays. From the apparently exponential portions of these decays, $k_1 = 25 \pm 15$ M^{-1} s^{-1} was found for substitution by isonicotinamide. Preliminary studies of the reaction of $Ru(edta)H_2O^{2-}$ with O_2 indicated the reaction to be moderately rapid ($k \sim 10^1$ M^{-1} s^{-1}) and kinetically well behaved. As it seemed possible that Ru(III) produced by residual dioxygen might be responsible for the ill-behaved Ru(II) substitution kinetics, studies of the Ru(III) reactions were undertaken.

The kinetics of the substitution reactions of $Ru(edta)(H_2O)^-$ ($pK_a(-CO_2H) = 2.37$, $pK_a(-OH_2) = 7.5$) were studied in buffered aqueous solutions (0.1 M acetate buffer (pH 5.5), $\mu = 0.2$ M KCF_3SO_3, 25 °C) by the stopped-flow technique. Absorbance increases due to complexation by L which was 10^{-4}–10^{-1} M and in at least tenfold excess over Ru(III) were monitored in the 300–400 nm range. Absorbance change vs. time curves were exponential for at least two half-lives. (Depending on L, a second stage—probably the addition of a second molecule of L—was responsible for up to 20% of the total absorbance change. As the apparent rate for this process was, in all cases, at least ten times slower than the first process, the two stages were readily disentangled.) Data for the first stage were found to conform to the equation $k_{obsd} = a + b[L]$ which is compatible with the approach to equilibrium

$$Ru(edta)(H_2O)^- + L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Ru(edta)L^- + H_2O \quad (2)$$

with $a = k_{-2}$, $b = k_2$ (see Figure 1). Values obtained for k_2/k_{-2} were in good agreement with K_2 determined from the magnitudes of the absorbance changes produced; that is, plots of ΔA^{-1} vs. $[L]^{-1}$, where ΔA is the absorbance increase accompanying the first stage of complexation by L, were linear with intercept/slope ratios which were within experimental error of the k_2/k_{-2} ratios. The rate constants k_2 were large and varied greatly with the nature of the entering ligand (ligand, k_2 (M^{-1} s^{-1})): CH_3CN, 30 ± 7; SCN^-, $(2.7 \pm 0.2) \times 10^2$; isonicotinamide, $(8.3 \pm 0.6) \times 10^3$; pyrazine, $(2.0 \pm 0.1) \times 10^4$.

In Figure 2 the rate constants k_2 obtained for substitution of L on $Ru(edta)H_2O^-$ are plotted vs. values for the ligand binding constant k_2/k_{-2}. The rate constants, which vary over three orders of magnitude, are seen to parallel the driving force for the substitution. The magnitude 0.95 of the slope of the plot (intercept 0.25) suggests a very high degree of bond making in the transition state[3] and so provides strong evidence for associative character in the substitution on Ru(III) in $Ru(edta)H_2O^-$. Although the displacement of NH_3 in $Ru(NH_3)_6^{3+}$ by nitric oxide also proceeds by an associative

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