

4, the other adducts, 5-7, afford new geometrical schemes. The extended linear O-bonded compound 5 deserved special mention since the two nitroxide  $\pi$  components are no longer parallel, as found in the centrosymmetric adducts but orthogonal ( $91.2(6)^\circ$ ) as shown in Figure 2. Owing to this orthogonality, a  $Rh\pi^*-NO\pi$  overlap cannot be responsible for the coupling of the nitroxide ligands. However, there is no symmetry limitation for the interaction of a Rh  $\sigma$  orbital with the two  $\sigma_z$  components of the nitroxide groups, and we suggest that, in all cases studied so far, it is this mechanism which is responsible for the magnitude of the observed couplings.

Further strong support for this mechanism comes from the magnetic behavior of the two remaining complexes 6 and 7. In these adducts, containing one or two axially bonded nitrogen atoms, owing to the near orthogonality of the nitroxide least-squares plane and the Rh-O<sub>4</sub> plane, the nitroxide  $\sigma_z$  components are nearly zero. Therefore, the large  $\pi$  component would give a large interaction with a Rh-Rh HOMO of  $\pi$  symmetry. With a Rh-Rh  $\sigma$  HOMO, the overlap is symmetry forbidden, and the nitroxyl-nitroxyl coupling is expected to be very weak (positive or negative) as observed.

The magnetic behavior of this series of rhodium-nitroxide complexes clearly demonstrates that the interligand interaction is highly geometry dependent. Although  $Rh\pi^*-NO\pi^*$  back bonding has been made responsible<sup>23</sup> for some of the observed properties of similar compounds, local symmetry considerations clearly show that the magnetic behavior of complexes 4-7 is better explained by a nitroxyl-nitroxyl coupling mechanism involving a  $\sigma$  Rh-Rh orbital.

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**Supplementary Material Available:** Listing of atomic positional parameters for compounds 4-7 (2 pages). Ordering information is given on any current masthead page.

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## Models for a Hypothetical Mechanism of Action of the Anticancer Agent Vinblastine

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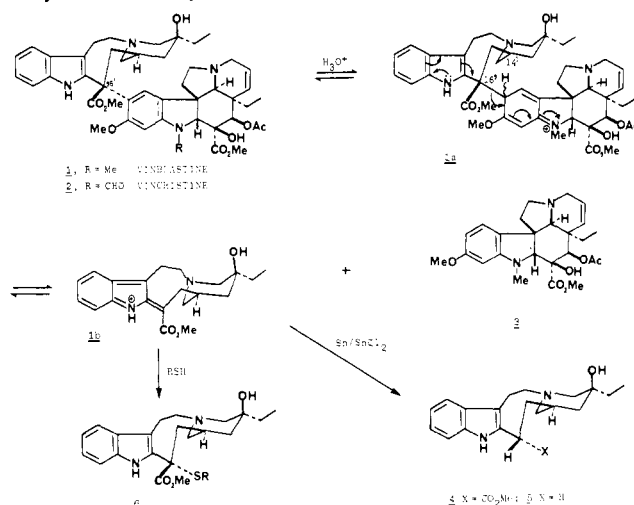
Vinblastine **1** and vincristine **2** are used in combination cancer chemotherapy for the treatment of a wide range of tumors.<sup>1</sup> While a large number of cytotoxic agents are commonly associated with DNA binding and/or intercalation and alkylation, thus inhibiting protein synthesis, there are no suggestions as to how vinblastine **1** might operate at the molecular level.<sup>2</sup> Apparently, vinblastine **1** and vincristine **2** bind to the protein tubulin and modify the accessibility of certain cysteine -SH groups, in particular two -SH groups, although it is not known which two.<sup>3</sup> It should be noted that a large number of antitumor agents have

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been shown to act by S-alkylation of an -SH function in target enzymes or coenzymes.<sup>4</sup>



In vitro, vinblastine **1** prevents the uptake of thymidine into DNA and uridine into RNA, processes that are dependent upon thymidylate synthetase, which utilizes -SH addition to C-6 (U).<sup>5</sup> Vinblastine **1** and vincristine **2** show markedly different toxicities. The former exhibits bone marrow depletion, whereas the latter is associated with neuropathy.<sup>6</sup> Given that the only difference between the two molecules is  $N^1$ -CH<sub>3</sub> **1** and  $N^1$ -CHO **2**, this substituent must exert a significant effect. Apparently, in vivo, vincristine undergoes considerably less metabolism than vinblastine.<sup>7</sup>

In this paper we present chemical evidence that vinblastine models can act as alkylating agents toward thiols. It is known that reductive cleavage of **1** with use of Sn/SnCl<sub>2</sub>/HCl gives vindoline **3** and velbanamine **5**, which arises from hydrolysis and decarboxylation of carbomethoxyvelbanamine **4**.<sup>8</sup> This process is best explained by a reversible ipso protonation<sup>9</sup> of vinblastine **1** to form the arenium ion **1a**, which can undergo fragmentation into the iminium ion **1b** and vindoline **3**. The iminium ion **1b** is reduced to carbomethoxyvelbanamine **4**.<sup>10</sup>

This degradation initiated the intriguing idea that under enzymatically controlled conditions vinblastine **1** can undergo ipso protonation to give **1a** and then **1b**, which can scavenge thiol groups to give adducts such as **6**. As a corollary to this, vincristine **2** is deactivated toward ipso protonation by the  $N^1$ -CHO group and cannot function as an alkylating agent.

When the vinblastine model **7**<sup>11</sup> was treated with aqueous TFA/*n*-BuSH/THF at 70 °C the adduct **9** (R = *n*-Bu) was isolated in 86% yield, along with *m*-methoxy-*N,N*-dimethylaniline. In a separate experiment **9** (R = *n*-Bu) was dissolved in neat TFA/*n*-BuSH/26 °C for 4.5 h, and the reduced product **10** was isolated in 68% yield. Exposure of **7** to concentrated HCl/*n*-

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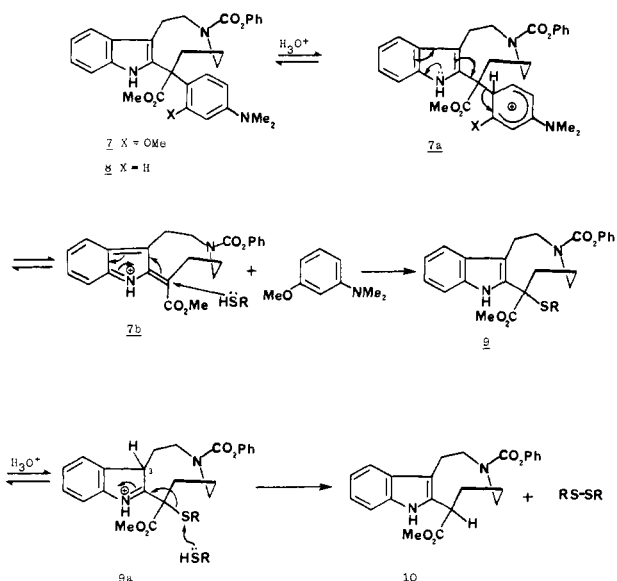
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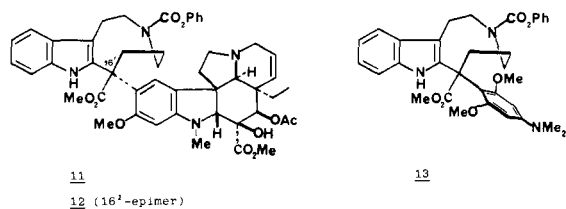
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(11) The synthesis of the model bis indole alkaloids **7**, **8**, **11**, **12**, and **13** will be described in detail later.



BuSH/MeOH at 65 °C lead directly to the reduced adduct **10** in 89% yield. It was predicted that the *des*-methoxy dimer **8**, which is less electron-rich, would be inert to the above ipso protonation conditions, and this indeed is the case. The reduction product **10** presumably arises from C-3 protonation of **9** to give **9a**, which is now an activated sulfenylating species. Thiol attack at sulfur on **9a**, followed by prototropic shift, gives the reduction product **10** and the disulfide.<sup>12</sup> When **9** (R = CH<sub>2</sub>Ph) was treated with PhCH<sub>2</sub>SH (2.0 equiv)/HBF<sub>4</sub> aqueous THF it gave **10** (69%) and dibenzyl disulfide (94% based upon **9**). In contrast, **9** (R = CH<sub>2</sub>Ph) on exposure to HBF<sub>4</sub>/aqueous THF gave dibenzyl disulfide (96%, based upon **9**), and no reduction product **10**. [PhCH<sub>2</sub>SH gave dibenzyl disulfide (18%) when treated with HBF<sub>4</sub>/aqueous THF.]<sup>13</sup> The bis indole alkaloid model **11** (natural configuration at C-16<sup>1</sup>) on treatment with aqueous TFA/*n*-BuSH/26 °C gave vindoline **3** (60%) and the reduction product **10** (41%). Similarly, **11** gave vindoline **3** (65%), (PhCH<sub>2</sub>S)<sub>2</sub>

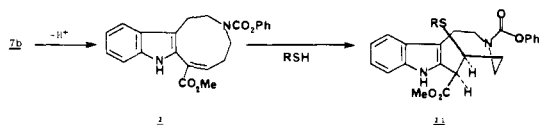


(43%), **9** (R = CH<sub>2</sub>Ph) (13%), and **10** (50%), when exposed to aqueous HBF<sub>4</sub>/THF/PhCH<sub>2</sub>SH/20 °C. Interestingly, the bis indole alkaloid model **12** (16<sup>1</sup>-epimer) gave vindoline **3** (40%), and no other identifiable fragments, when exposed to the above conditions.

Vinblastine itself could be cleaved with HCl (12 M)/*n*-BuSH to give 4-deacetylvindoline, but no identifiable products from the top half.<sup>14</sup>

To illustrate that the more susceptible the bottom half of the bis alkaloids are to ipso protonation the more readily they are cleaved, we treated the *m,m*-dimethoxy analogue **13** with vindoline

(12) If **9** were to reverse to **7b**, this intermediate should undergo proton loss to give the  $\alpha,\beta$ -unsaturated ester **i**, which would subsequently conjugatively add RSH to give the adduct **ii**. We have made **i**, and it is not formed (nor **ii**) when **9** is exposed to RSH/H<sup>+</sup>.



(13) The amount of dibenzyl disulfide produced in the cleavage reaction approximately corresponds to the amount of reduction to give **10**.

(14) We would have expected to isolate **4** or **5**. Although it should be noted that the original Sn/SnCl<sub>2</sub> cleavage of **1** only gave **5** in very low yields.<sup>8</sup>

**3**/aqueous TFA/THF at 25 °C for 52 h and isolated **11/12** (ca. 1:1) in 24% yield.

The acid-promoted cleavage of the model bis alkaloid **7**, subsequent iminium ion **7b** thiol trapping, and eventual reductive cleavage provide an interesting prediction. There could be a biological difference between **7** and **8**, since only **7** can produce **7b**. It turns out that **7** is weakly cytotoxic, whereas **8** is not.<sup>15</sup> While this does not in anyway necessarily corroborate the mechanistic hypothesis, it is nevertheless provocative. The specific acidic conditions used to generate **7b** in no way represent so-called physiological conditions, but the exemplary ability of enzymes to lower  $\Delta G^\ddagger$  could overcome this problem.

The above hypothesis may be useful in explaining the *in vivo* biological and pharmacological properties of bis alkaloids and for designing new drugs based upon natural bis alkaloids.<sup>16</sup>

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(15) **7** has a CEM IC 50 4.3  $\mu\text{g}/\text{mL}$ , whereas **8** > 20.

(16) Vincristine **2** should not undergo reductive cleavage. *N*<sup>1</sup>-Formyl-6,7-dihydro-16-methoxytaberionine does not undergo the Potier coupling reaction (see ref 10).

### Conformationally Dependent Intrinsic and Equilibrium Isotope Effects in *N*-Methylpiperidine

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Conformational equilibrium isotope effects (CEIE)<sup>1</sup> have recently been shown to differ substantially between cyclohexane, where deuterium in a CHD group prefers the equatorial over the axial position by  $6.3 \pm 1.5$  cal/mol,<sup>2</sup> and 5,5-dimethyl-1,3-dioxane-2-*d*<sub>1</sub>, where the CHD lies between two oxygen atoms and deuterium prefers the equatorial position by  $49 \pm 3$  cal/mol.<sup>3</sup> Anet and Kopelovich attributed the difference primarily to  $n-\sigma^*$  (negative) hyperconjugation which weakens and lengthens the bond to an axial substituent that is anti to a lone electron pair.<sup>3</sup> Until their observation, there had been little experimental evidence for the predicted angular dependence of the energetic consequences of negative hyperconjugation.<sup>4,5</sup> We now report an even larger CEIE in *N*-methylpiperidine and also report a substantial difference in the *intrinsic* isotope effect on the <sup>15</sup>N chemical shift

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