thus prepared was oxidized to nitrogen gas with sodium hypobromite *in vacuo* as described by Bremner.[6] Isotope ratio measurements were made on a Nuclide RMS 6-60 isotope ratio mass spectrometer relative to a tank nitrogen standard. Isotope effects were calculated as described previously.[5] The results of six determinations of the isotope effect are summarized in Table I. The isotope ratios given in this table

Table I. Nitrogen Isotope Effects on the Chymotrypsin-Catalyzed Hydrolysis of *N*-Acetyl-L-tryptophanamide at pH 8.0, 25°

% reaction[a]	—Isotope ratios[b] × 10^6— Low conversion	100% conversion	k^{14}/k^{15}
11.9	9261	9333	1.0091
10.5	9274	9357	1.0095
10.0	9244	9349	1.0119
9.3	9257	9355	1.0111
9.6	9241	9335	1.0106
9.6	9265	9349	1.0095
		Mean	1.010 ±0.001

[a] Determined by spectrophotometric monitoring of the reaction at 306 mμ. 100% reaction was found to correspond to $\Delta\epsilon = 70$ M^{-1} cm^{-1}. [b] Decade settings for the ratio *m/e* 29/28, corrected to tank standard = 9200.

are not actual isotopic abundances, but are instead corrected decade settings on the isotope-ratio mass spectrometer. The decade settings are directly proportional to isotopic abundances and can be used directly in calculating isotope effects. The correctness of these results was indicated by several tests. (1) The isotope effect was constant from experiment to experiment. (2) Standard ammonia samples could be carried through our procedure and analyzed with a reproducibility of ±0.000002. (3) No ammonia was found after steam distillation and concentration of a reaction solution if either enzyme or substrate was omitted. (4) The isotope ratios for the 10% samples and for the 100% samples were constant for all experiments.

Of the three or more steps involved in the acylation of chymotrypsin by *N*-acetyl-L-tryptophanamide, only the rate of the step in which the carbon–nitrogen bond is broken is expected to be affected appreciably by isotopic substitution.[7] A nitrogen isotope effect will be observed in this reaction only if the steps leading from the carbon–nitrogen cleavage step back to the starting materials are not slow compared to the cleavage step.[5] The nitrogen isotope effect observed in this case is similar to those observed in other cases where carbon–nitrogen single bond breaking occurs in the rate-determining step[8] and indicates that the above condition in fact obtains—that is, the carbon–nitrogen bond-breaking step is rate determining.

(6) J. M. Bremner, in "Methods of Soil Analysis," American Society of Agronomy, Madison, Wis., 1965, p 1256.

(7) There might also be a small isotope effect on some other step due to the loss of the extra zero-point energy associated with the partial double bond character of the amide carbon–nitrogen bond. Such might be the case, for example, if a tetrahedral intermediate is formed during the reaction. However, until further information is available about the structures of the intermediates involved in this reaction, we are unable to estimate the importance of such an effect.

(8) P. J. Smith and A. N. Bourns, *Can. J. Chem.*, **48**, 125 (1970); G. Ayrey, A. N. Bourns, and V. A. Vyas, *ibid.*, **41**, 1759 (1963); S. Seltzer and S. G. Mylonakis, *J. Amer. Chem. Soc.*, **89**, 6584 (1967).

If we assume that the rate constants for the chymotrypsin-catalyzed hydrolysis of *N*-acetyl-L-tryptophanamide are approximately the same as those of the very similar substrate furylacryloyltryptophanamide, our results show that the slow step in the kinetic scheme of Hess, *et al.*,[2] is, as they suggested, the step in which the carbon–nitrogen bond is broken.[9] This conclusion is also consistent with a number of previous observations concerning the chymotrypsin-catalyzed hydrolysis of specific substrate amides, for example, the large substituent effect on both the rate and the solvent isotope effect in the hydrolysis of acetyltyrosine anilides.[3] Such an effect is most easily explicable if carbon–nitrogen bond cleavage occurs in the slow step of the reaction.[10]

Acknowledgment. This research was supported by grants from the National Institutes of Health (NS-07657), the University of Wisconsin Graduate School, and the Research Corporation.

(9) Our results do not exclude the possibility that there might be more intermediates in this reaction than have been observed. Such a possibility would not change our conclusion that the carbon–nitrogen bond breaking is occurring in a slow step.

(10) The observation that the solvent isotope effect in this series of compounds varies from 1.5 to 2.8 with various substituted anilides lays to rest forever the objection that has repeatedly been raised that solvent isotope effects in enzymatic reactions may merely be indicators of solvent-sensitive enzyme conformational changes, rather than of transition state-structure and solvation. The former factor should be nearly constant for various substrates, and therefore can have a maximum value of 1.5 for this series of compounds. The remaining isotope effect (up to a factor of 2) must be due to the second factor.

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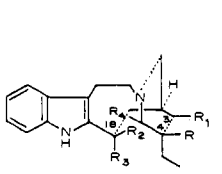
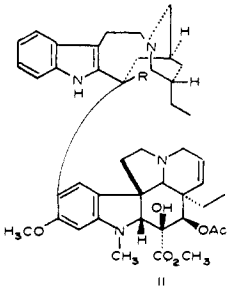
Studies on the Synthesis of Monomeric and Dimeric Vinca Alkaloids. The Total Synthesis of Isovelbanamine, Velbanamine, Cleavamine, 18β-Carbomethoxycleavamine, and Catharanthine

Sir:

Previous publications from our laboratory have demonstrated the general utility of the chloroindolenines of the cleavamine derivatives (I, R = R_1 = R_2 = R_3 = R_4 = H and R = R_1 = R_2 = R_4 = H; R_3 = $COOCH_3$) in the synthesis of monomeric[1,2] and dimeric[3] indole and dihydroindole alkaloids.





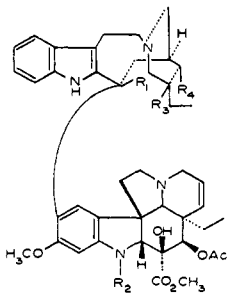
II

(1) J. P. Kutney, W. J. Cretney, P. Le Quesne, B. McKague, and E. Piers, *J. Amer. Chem. Soc.*, **88**, 4756 (1966).

(2) J. P. Kutney, W. J. Cretney, P. Le Quesne, B. McKague, and E. Piers, *ibid.*, **92**, 1712 (1970).

(3) J. P. Kutney, J. Beck, F. Bylsma, and W. J. Cretney, *ibid.*, **90**, 4504 (1968).

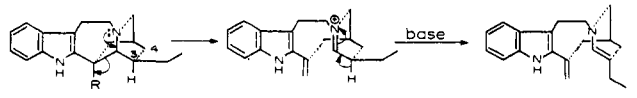
Of particular relevance to the work presented here was the realization that the dimeric systems obtained[3] (II, R = H and $COOCH_3$) were close relatives of the naturally occurring Vinca alkaloids, vinblastine (III),[4,5] vincristine (IV),[4,5] leurosidine (V),[6] and leurosine (VI),[7,8] several of which are potent antitumor agents.



	R_1	R_2	R_3	R_4
III	CO_2CH_3	CH_3	OH	H
IV	CO_2CH_3	CHO	OH	H
V	CO_2CH_3	CH_3	H	OH
VI	CO_2CH_3	CH_3		

In order to achieve a laboratory synthesis of these medicinally important substances as well as provide a family of closely related derivatives for biological evaluation, it was now desirable to synthesize the appropriate indole intermediate whose functionality could be varied in the manner required by the above alkaloids (III–VI). We wish now to report the successful achievement of this goal.

Dihydrocatharanthine (VII, R = CO_2CH_3), a compound available in racemic form from a previous total synthesis[2] or in optically active form *via* catalytic reduction of the alkaloid catharanthine,[9] was chosen as the starting material. The optically active isomer was reduced with lithium aluminum hydride and the resulting alcohol VII (R = CH_2OH) was obtained. This molecule possesses the required stereochemistry to undergo an interesting fragmentation reaction and thereby provide a suitably functionalized cleavamine analog.



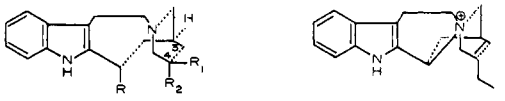
VII VIII

Indeed treatment of the tosylate with triethylamine[10] resulted in the expected ring opening and concomitant displacement of the tosyl group to give the seco-diene VIII, $C_{20}H_{24}N_2$, mp 136–136.5°, in 62% overall yield based on the alcohol. This substance exhibited the following spectral properties: λ_{max}^{MeOH} mμ (log ε) 306 (4.16), 340 (sh, 3.49); ν_{max}^{KBr} 1657 cm^{-1} (C=C—N<); nmr τ 4.29 (broad singlet, 1 H, >N—*CH*=C) and 4.75 and 4.89 (singlets, 2 H, >C=CH_2).[11]

Reaction of VIII with osmium tetroxide led to the hydroxylation of both the enamine and exocyclic double bonds to give the tetrol I (R = R_2 = R_4 = OH; R_1 = H; R_3 = CH_2OH), $C_{20}H_{28}N_2O_4$, mp 120–123°. This compound now possessed a typical indole uv spectrum and the following nmr signals: τ 5.42 (broad singlet, 1 H, >N—*CH*OH) and 6.36 (broad singlet, 2 H, sharpened on D_2O exchange, –CH_2OH). Hydrogenolysis (sodium borohydride) of the carbinol amine hydroxyl function converted the tetrol to the triol I (R = R_2 = OH; R_1 = R_4 = H; R_3 = CH_2OH), mp 230–235° dec, in essentially quantitative yield. The vicinal diol portion of this molecule was then cleaved with periodate to provide the ketol I (R = OH; R_1 = R_4 = H; R_2, R_3 = O), $C_{19}H_{24}N_2O_2$, mp 105–109° dec; λ_{max}^{MeOH} mμ (log ε) 238 (4.16), 317 (4.25); ν_{max}^{Nujol} 1615 cm^{-1}.

Further elaboration of the ketol to suitably functionalized cleavamine derivatives could be conveniently conducted by controlled reaction conditions. Thus mild reduction (sodium borohydride, room temperature) gave the diol I (R_1 = R_2 = R_4 = H; R = R_3 = OH; $C_{19}H_{26}N_2O_2$; mp 195–200° dec; nmr τ 4.28 (doublet of doublets, J = 2 and 10 Hz, 1 H, C_{18}–H)) while more vigorous conditions (lithium aluminum hydride in refluxing *N*-methylmorpholine) removed the C_{18} hydroxyl function, and the resultant crystalline product I (R = OH; R_1 = R_2 = R_3 = R_4 = H), mp 190–194°, was isomeric with the known compound, velbanamine, obtained from degradation of vinblastine.[4] On this basis, this synthetic intermediate was called isovelbanamine.

Isovelbanamine not only provided the desired C_4 epimer for further synthetic studies in the dimeric series but it represented a key intermediate in completing the total synthesis of cleavamine (IX, R = H; R_2 = CH_2CH_3; 3,4 double bond), 18β-carbomethoxycleavamine (IX, R = $COOCH_3$; R_2 = CH_2CH_3; 3,4 double bond), and velbanamine (IX, R = H; R_1 = CH_2CH_3; R_2 = OH). Acid-catalyzed dehydration (concentrated sulfuric acid) of isovelbanamine provided cleavamine, identical with an authentic sample (tlc, superimposable ir and nmr) while reaction with aqueous sulfuric acid yielded velbanamine.[12]



IX X

On the basis of our earlier work[1,2,13,14] a total synthesis of catharanthine (VII, R = $COOCH_3$, 3,4 double bond), a major alkaloid isolated from *Vinca rosea Linn*, starting with cleavamine could be readily achieved. Cleavamine was oxidized with *tert*-butyl hypochlorite to form a chloroindolenine which upon reaction with sodium acetate in glacial acetic acid allowed conversion to the stable quaternary salt, X. Treatment of the latter with potassium cyanide in dimethylformamide yielded a crystalline product, mp

(4) N. Neuss, M. Gorman, W. Hargrove, N. J. Cone, K. Biemann, G. Buchi, and R. Manning, *J. Amer. Chem. Soc.*, **86**, 1440 (1964).
(5) J. W. Moncrief and W. N. Lipscomb, *ibid.*, **87**, 4963 (1965).
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(8) D. J. Abraham and N. R. Farnsworth, *J. Pharm. Sci.*, **58**, 694 (1969).
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(10) U. Renner, K. A. Jaeggi, and D. A. Prins, *Tetrahedron Lett.*, 3697 (1965).
(11) Satisfactory elemental analyses and high-resolution mass spectra were obtained on all new compounds reported. All nmr spectra were recorded at 100 MHz on a Varian HA100 spectrometer.
(12) We are grateful to Dr. N. Neuss, Lilly Research Laboratories, Indianapolis, Ind., for providing us with an authentic sample of this substance for purposes of comparison.
(13) J. P. Kutney, R. T. Brown, and E. Piers, *J. Amer. Chem. Soc.*, **86**, 2286, 2287 (1964).
(14) J. P. Kutney, R. T. Brown, E. Piers, and J. R. Hadfield, *ibid.*, **92**, 1708 (1970).

87–90°, which proved to be 18β-cyanocleavamine (IX, R = CN; R_2 = CH_2CH_3; 3,4 double bond). The overall yield was 32% based on starting cleavamine. Hydrolysis and esterification of the latter intermediate gave the known[15] 18β-carbomethoxycleavamine (IX, R = $COOCH_3$; R_2 = CH_2CH_3; 3,4 double bond). The transannular cyclization of this compound to provide catharanthine[14] now completes the total synthesis of this alkaloid.

In summary, the above work describes a versatile synthetic pathway to essentially all the indole units necessary for the synthesis of dimeric Vinca alkaloids. Apart from the dimeric systems which are available *via* the chloroindolenine approach mentioned above, the C_{18}-hydroxy derivatives can also provide dimers from the reaction sequence established by Buchi[16] and more recently applied by Harley-Mason.[17] We hope to present results in these various directions in future publications.

Finally, we wish to mention that the syntheses of velbanamine[18,19] and catharanthine[19,20] have also been reported from other laboratories in which completely different routes have been employed.

Acknowledgment. Financial aid from the National Research Council of Canada and the National Cancer Institute of Canada is gratefully acknowledged.

(15) J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, and V. R. Nelson, *J. Amer. Chem. Soc.*, **92**, 1704 (1970).
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(18) G. Buchi, P. Kulsa, and R. L. Rosati, *J. Amer. Chem. Soc.*, **90**, 2448 (1968).
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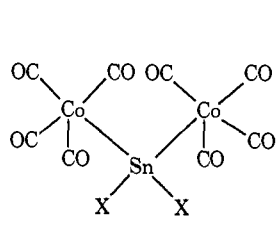
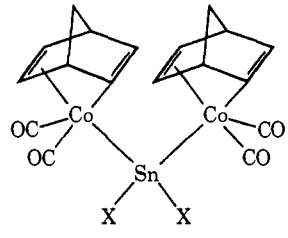
Structure and Activity in a Multicenter π-Complex Reaction

Sir:

We report the isolation and structure of model intermediates in a π-complex multicenter reaction,[1,2] and propose a relationship between the electronic structure and the selectivity of the catalytic species.





Ia, X = Cl Ib, X = Br or I III, X = Cl
IIa, X = C_6H_5 IIb, X = CH_3 IV, X = C_6H_5

The heterometallic cobalt carbonyls I[3] and II[4] were prepared. These compounds, analogous in structure to catalysts described by Schrauzer and coworkers,[1,2] were also found to catalyze the dimerization of norbornadiene. However, the stereospecificity is markedly sensitive to the substituent X; at 60° Ia gives exclusively the bisnortricyclene known as Binor-S,[1,2,5,6] proved below to be the syn isomer *VI*, while *IIa* gives predominantly the exo-trans-exo isomer[2] in addition to three dimers other than Binor-S. From the residues of the respective reaction mixtures we were able to isolate dark-red crystals of the (catalytically active) π-complexes III and IV believed to be intermediates in these reactions.

The structures of III and IV have been determined by X-ray diffraction. Though not isomorphous, both compounds crystallize in space group $P2_1/c$ with four molecules per unit cell. Lattice parameters[7] are: $Cl_2Sn(Co(CO)_2C_7H_8)_2$ (III), a = 18.302 (16), b = 11.838 (13), c = 16.222 (8) Å, β = 143.61 (8)°; and $(C_6H_5)_2Sn(Co(CO)_2C_7H_8)_2$ (IV), a = 9.310 (11), b = 17.279 (27), c = 20.229 (30) Å, and β = 123.06 (5)°. The intensities of 4741 (III) and 2971 (IV) unique reflections were measured on a four-circle diffractometer with Mo Kα radiation. Patterson and Fourier methods were used to solve the structures, and refinement by full-matrix least squares gave conventional R factors of 0.048 (III) and 0.078 (IV) for the 2977 (III) and 1965 (IV) reflections, respectively, above background. Hydrogens were not located; the refinement of III assumed anisotropic thermal motion for all atoms, but for IV the carbon and oxygen atoms were restricted to isotropic temperature factors.

The bond angles at cobalt, essentially identical in III and IV, are those of a distorted trigonal bipyramid, if the C=C bonds are regarded formally as single ligands (Figure 1). Both carbonyls are equatorial, tin is axial, and the norbornadienes, which are π bonded to cobalt through the endo lobes of the C=C bonds, each span one equatorial and one axial site. However, the ‖—Co—‖ angles, which range between 71.3 and 72.8° for the four independent cobalt centers, are substantially less than the 90° of an ideal trigonal bipyramid. This orientation permits the olefin–metal bonds, defined by lines drawn from the cobalts to the midpoints of the associated C=C bonds, to be nearly perpendicular (±3°) to the nodal planes of the olefin moieties.

There are, however, significant structural differences between III and IV. These differences can be explained[4,8] by the second-order hybridization effect proposed by Bent,[9] which predicts concentration of p character from the tin atom in bonds to electronegative substituents and the freeing of s character for the metal–metal bonds; in fact, these structures provide some of the most convincing evidence[8] to date for the importance of this hypothesis in compounds with metal–

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(3) D. J. Patmore and W. A. G. Graham, *Inorg. Chem.*, **5**, 1405 (1966).
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