A HIGHLY EFFICIENT AND COMMERCIALLY IMPORTANT SYNTHESIS OF THE ANTITUMOR CATHARANTHUS ALKALOIDS VINBLASTINE AND LEUROSIDINE FROM CATHARANTHINE AND VINDOLINE

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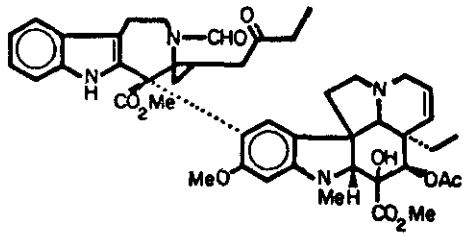
Abstract - Extensive studies of various reaction parameters for the chemical coupling of catharanthine (1) and vindoline (2) followed by evaluation of highly unstable intermediates by careful reaction control have provided essentially a quantitative yield in this coupling reaction. Subsequent regioselective reduction of the resultant intermediate 3 by select NADH models affords a high yield of an enamine (5) which, without isolation, is selectively oxidized to unstable iminium intermediates 6 and 7 and the latter, again without isolation, are finally reduced to vinblastine (8, 40%), leurosidine (10, 16%), and 3',4'-anhydrovinblastine (4, 12%). The entire process of five steps (1 → 11 + 2 → 3 → 5 → 6,7 → 8,10,4) can be achieved in a one-pot operation and the high overall yield of vinblastine (8) requires that each reaction must proceed in yields in excess of 80%.

Our success in the biomimetic conversion of 3',4'-anhydrovinblastine (4) to the important anti-cancer drug, vinblastine (8), as described in the preceding publication[1], has prompted us to investigate the adaptation of such a synthetic sequence towards an efficient synthesis of 8, and in a manner which may be applied subsequently for industrial production (Scheme 1). The important features of the above biomimetic synthesis include: (a) the established pivotal role of the conjugated iminium intermediate 3 between the two monomeric alkaloids, catharanthine (1) and vindoline (2), and the vinblastine family of bisindole alkaloids; (b) regioselective 1,4-reduction of 3 to the key enamine intermediate 5 by β-NADH;

(c) effective oxygenation of 5 to a vinblastine precursor 6, and (d) borohydride reduction of the latter to yield vinblastine (8).

The main drawbacks of the above process are the relatively low overall yield of 8 from 4 (23%) and the requirement of excess costly β-NADH for the reduction of 3 to 5. The main reasons for the low yield of 8 are the formation of vinamidine (9) due to hydrolysis of the iminium intermediates 6 and/or 7 and non-exclusive regiospecific 1,4-reduction of 3 thereby resulting in some regeneration of 4 (20%). To minimize hydrolysis of the iminium intermediates we prepared 3 (>85%) directly under anhydrous conditions by the modified Polonovski coupling reaction of catharanthine (1), via its N-oxide (11), with vindoline (2)[2]. Anhydrous conditions were also maintained in subsequent steps by using NADH models for the 1,4-reduction of 3 to 5. Various NADH models (12-21) were studied for the regioselective reduction. We found that N-substituted 1,4-dihydronicotinamides possessing functional groups such as ester or carboxylic acid, capable of coordination with the iminium ion (3), to be the most effective in producing the enamine 5. Results are listed in Table 1.



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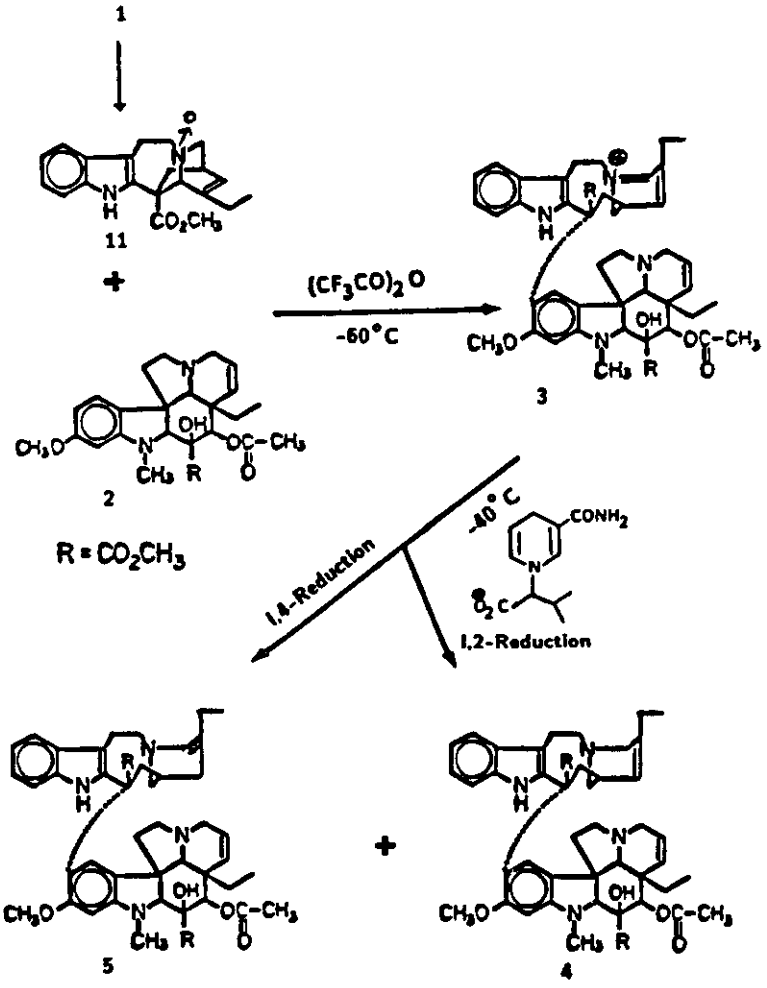
Because of the more ready separation of 18 (and its oxidized form) in the work-up process, 18 was selected for further optimization studies. Of the various parameters examined, lowering the temperature of the reduction was most effective in improving the yield of enamine 5. Table 2 shows that at -40° C a ratio of 4.2:1 in favor of the 1,4-reduction product 5 was obtained. Further reduction of 5 to the corresponding saturated derivatives (4'-deoxy-leurosidine and 4'-deoxyvinblastine) was also suppressed. The yield of the combined products, 5 and 4, also increased to 85%. These results represent an overall improvement on the regioselective 1,4-reduction of 3 when compared with that by β-NADH as described in the preceding publication[1].

Scheme 1. A Highly Efficient "One Pot" Process for the Synthesis of Vinblastine (8) and Leurosidine (10) from Catharanthine (1) and Vindoline (2).



Scheme 1 (continued)

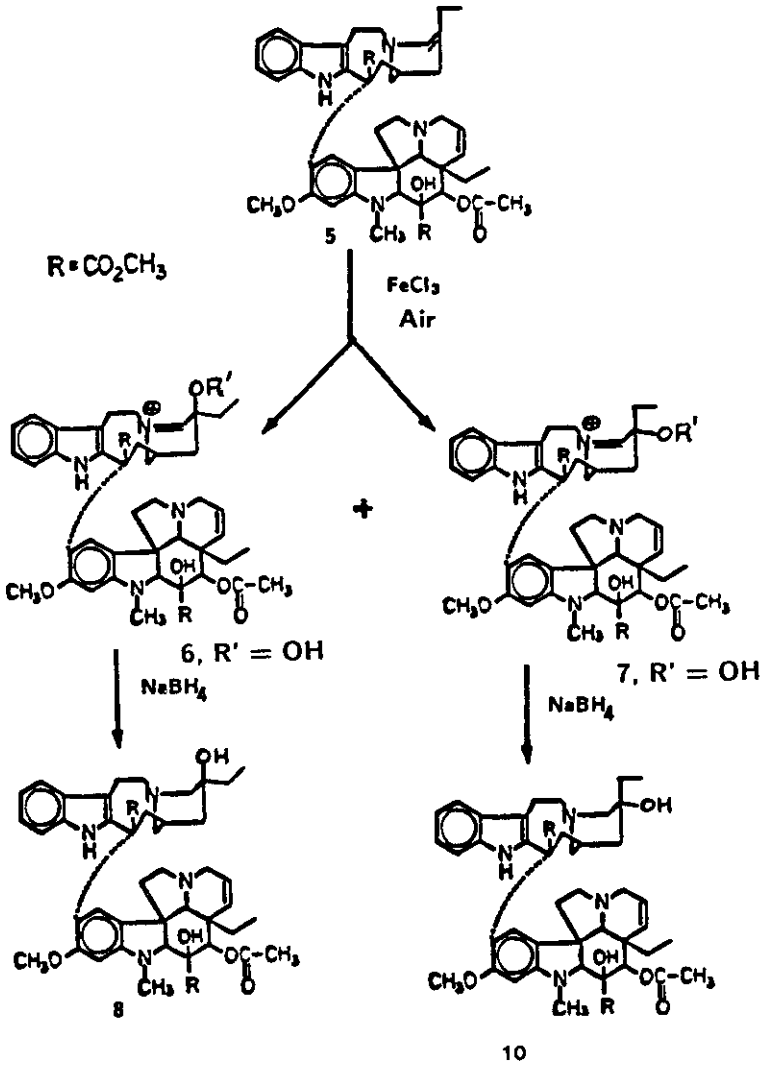
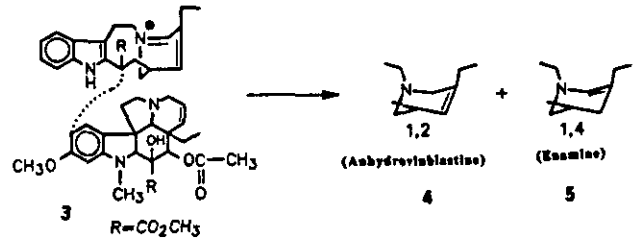
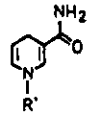
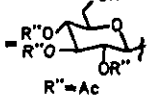
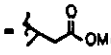
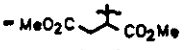
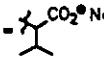
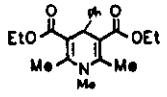
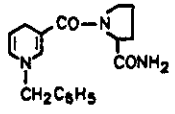


Table 1. NADH Models for 1,2- Versus 1,4-Reduction of Iminium Intermediate (3) at 20° C.[a]



3 R=CO_2CH_3 4 5

	Reducing Agents	Reduction Products[b] 1,4 (5) : 1,2 (4)	Combined Yield of 1,2 + 1,4 Reduction Product (%)
			
12	R' = $CH_2C_6H_5$	1 : 1	75
13	= $CH(C_6H_5)_2$	0.9 : 1	60
14		2 : 1	65
15	 R''=Ac	3[c] : 1	70
16		1.1 : 1	65
17		2.3 : 1	70
18		2.2 : 1	70
19		0.4 : 1	60
20		1 : 1	40
21		1.1 : 1	60

[a] Typical procedure:- 100 mg 3 in MeOH (6 ml) at 20° C to which reducing agents 12-21 (1-6 eq.) in MeOH (6 ml) were added.

[b] HPLC quantitation

[c] Reduction rate very slow, prolonged reaction resulted in significant further reduction of 5 to the saturated derivatives (50%)

Table 2. Effect of Temperature on 1,4- vs. 1,2-Reduction of Iminium 3 by 18[a]

Temp. (° C)	1,4-:1,2-Reduction Products[b] (5):(4)	Yield[c] (%)
20	2.2:1	70
-20	3.2:1	80
-40	4.2:1	85

[a] 6 equiv. of 18, reaction monitored by HPLC to completion.
[b] Quantitation by HPLC.
[c] Combined 1,2-reduction (3',4'-anhydrovinblastine, 4) + 1,4-reduction (enamine, 5) products. Yield represents overall yield and is based on vindoline utilized.

Results of oxidation studies of the enamine 5 with various reagents are shown in Table 3.

Table 3. Oxidation of Enamine 5[a] with Various Oxidizing Systems

Oxidizing System	Conditions[b]	Yield (%)[c] Vinblastine (8)	Leurosidine (10)
FMN/air	1-3 equiv., 25° C, 0-3 h	-	-
$FeCl_2/H_2O_2$	1.2-2 equiv., $FeCl_2$; 3-20 equiv. H_2O_2	-	-
$FeCl_3/H_2O_2$	8 equiv. $FeCl_3$; 3-20 equiv. H_2O_2	2	-
$CuCl_2$/air[d]	1 equiv., 0° C, air, 30-120 min	-	-
$Fe_2(acac)_3$/air[d]	1 equiv., 0° C, 5-10 min	6	-
$FeCl_3$/air[d]	1 equiv., 0° C, 5-10 min	14	10

[a] Reaction mixture containing 5 and 4 obtained by reduction of 3 with 18 at -40° C was used directly.
[b] All oxidations were carried out in the dark.
[c] HPLC quantitation after reductive work-up with $NaBH_4$. Yield represents overall yield and is based on vindoline utilized.
[d] Dry air was bubbled through the solution at 60 ml/min. Pure O_2 was also used, with little difference in yields of 8 and 10.

Direct aeration of 5 in the presence of $FeCl_3$ was found to be the most effective in introducing the desirable oxygenation at the C4' position of 5. It was necessary to carry out a reductive work-up of the resulting mixture with $NaBH_4$ in order to obtain 8 and 10. This implied that the $FeCl_3$/air oxidation of 5 resulted in the initial formation of two epimeric iminium intermediates 6 and 7 similar to those described in the preceding publication[2]. Borohydride reduction of the two intermediates 6 and 7 would lead to 8 and 10 respectively.

Ferric chloride was essential for the desirable oxygenation of 5 at C4'. As shown in Table 4 no vinblastine (8) was obtained in the absence of $FeCl_3$. Two equivalents of $FeCl_3$ gave the best yield of 8 and this condition was used in all subsequent optimization experiments to evaluate other reaction parameters.

Table 4. Effect of Ferric Chloride on Production of Vinblastine (8) from Enamine 5[a].

Amount of $FeCl_3$ (Equivalents)	Yield of Vinblastine (8) (%)[b]
0	0
1	13.3
2	19.0
3	10.4

[a] Reaction mixture containing 5 and 4 obtained by reduction of 3 with 18 at -40° C was used directly. Reaction conditions: air bubbled through the solution at 60 ml/min for 5-10 min at 0° C in the dark.

[b] By reverse-phase HPLC quantitation, after reductive work-up with $NaBH_4$. Yield represents overall yield and is based on vindoline utilized.

Table 5 shows the effect of varying the length of the $FeCl_3$/air oxidation of 5 on the yield of 8. Depending on the scale of reaction, 10 to 20 min of reaction time appeared to be adequate.

Table 5. Effect of Time of Oxidation on Production of Vinblastine (8) from Enamine 5[a].

Time (min)	Yield of Vinblastine (8) (%)[b]
1	8.2
5	15.4
10	15.5
15	15.7
45	6.5

[a] Reaction mixture containing 5 and 4 obtained by reduction of 3 with 18 at -40° C was used directly. Reaction conditions: - 2 equiv. ferric chloride added, air bubbled through the solution at 60 ml/min at 0°C in the dark.

[b] By reverse-phase HPLC quantitation after reductive work-up with $NaBH_4$. Yield represents overall yield and is based on vindoline utilized.

As shown in Table 6, the most effective temperature for the $FeCl_3$ air oxidation of 5 on the production of 8 is at 0° C to 20° C.

Table 6. Effect of Oxidation Temperature on Production of Vinblastine (8) from Enamine 5[a].

Temp., 0° C	Yield of Vinblastine (8) (%)[b]
-40	3.7
-23	6.2
0	19.6
20	20.6
45	16.0

[a] Reaction mixture containing 5 and 4 obtained by reduction of 3 with 18 at -40° C was used directly.
Reaction conditions: 2 equiv. ferric chloride added, air bubbled through the solution at 60 ml/min for 15 min in the dark.

[b] By reverse-phase HPLC quantitation after reductive work-up with $NaBH_4$. Yield represents overall yield and is based on vindoline utilized.

Table 7. Effect of Dilution on Production of Vinblastine (8) from Enamine 5[a].

Dilution Factor[c]	Yield of Vinblastine (8) (%)[b]
1	19.6
5	25.2
10	30.1
20	29.6
50	24.7

[a] Reaction time containing 5 and 4 obtained by reduction of 3 with 18 at -40° C was used directly.
Reaction conditions: 2 equiv. ferric chloride added, air bubbled through the solution at 60 ml/min for 15 min at 0° C in the dark.

[b] By reverse-phase HPLC quantitation after reductive work-up with $NaBH_4$. Yield represents overall yield and is based on vindoline utilized.

[c] Dilution Factor 1 - 3 (100 mg) in 6 ml methanol to which 18 (6 equiv.) in 6 ml methanol was added. (Total volume = 12 ml) Dilution Factor 5 = Total volume of 60 ml, by addition of MeOH (48 ml) before addition of $FeCl_3$, etc.

The concentration of enamine 5 for the $FeCl_3$/air oxidation was found to have a significant effect on the final yield of vinblastine (8). Results as indicated in Table 7, showed that on dilution of the enamine solution by a factor of 10 to 20 times before oxidation with $FeCl_3$/air, the yield of 8 increased by almost 50%. Subsequently, by incorporating all the above optimized conditions and on scaling up the reaction of catharanthine (1, 500 mg) and vindoline (2, 450 mg) for the initial coupling to prepare 3, the overall yield of 8 was

improved to 40% (isolated) based on the monomeric alkaloids. In addition, leurosidine (10) and 3',4'-anhydrovinblastine (4) were isolated in 16% and 12% yield respectively.

For practical purposes and possible commercial application, we have incorporated all the above synthetic steps into an overall process conducted in a 'one-pot' operation from catharanthine (1) and vindoline (2) (Scheme 1). Isolation of the various intermediates (3, 5, 6, 7) is not necessary and an efficient synthesis of the anti-cancer drugs vinblastine (8) and leurosidine (10) is available. Previous syntheses of (8) and (10) were of poor overall yields from the monomeric alkaloids (1) and (2)[3]. The use of toxic reagents such as thallium triacetate and osmium tetroxide also made these syntheses less desirable.

It should be noted that in this overall process involving five distinct and separate chemical reactions in a 'one-pot' operation, an overall yield of 40% for 8 requires that each reaction within the sequence must proceed with yields in excess of 80%. Indeed when yields of the additional bisindole products 10 and 4 are considered (overall yield of 68%), it is clear that several of these reactions, for example, coupling of 1 and 2 to produce 3, must proceed in quantitative yield. Methods of scale-up and development of technology for a future commercial process are presently under study.

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REFERENCES

1. J. P. Kutney, L. S. L. Choi, J. Nakano, and H. Tsukamoto. 1988. Accompanying publication.
2. J. P. Kutney, T. Hibino, E. Jahngen, T. Okutani, A. H. Ratcliffe, A. M. Treasurywala, and S. Wunderly. Helv. Chim. Acta, 1976, 59, 2858.
3. P. Mangeney, R. Z. Andriamialisoa, N. Langlois, Y. Langlois, and P. Potier. J. Am. Chem. Soc., 1979, 101, 2243.

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