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PRODUCTION OF 3', 4'-ANHYDROVINBLASTINE: A UNIQUE CHEMICAL SYNTHESIS

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ABSTRACT. Preliminary investigations have led to the discovery that ferric ion can couple catharanthine and vindoline, in aqueous acidic media, to produce 3',4'-anhydrovinblastine as the major product. A conversion of 77% could be realized under optimized conditions.

Alkaloids derived from plants of the Apocynacae family have long been known for their medicinal properties (1,2). Of these, the Vinca alkaloids vinblastine (VLB) and vincristine (VCR), isolated from the leaves of Catharanthus roseus, are proven chemotherapeutics in the treatment of various carcinomas (1,3-5). However, these compounds comprise only a minute fraction of the overall alkaloid profile (6,7) thereby complicating isolation and concomitantly, limiting availability of these anti-tumour agents. Such shortcomings have spurred numerous investigations into possible synthetic routes of production (8).

In comparison with the dimeric products, the Aspidosperma (vindoline) and Iboga (catharanthine) constituents are relatively more plentiful; thus, rather than attempting the more arduous total chemical synthesis, many groups (9-14) have concentrated on the coupling reaction leading to the production of an intermediate in VLB and VCR synthesis, 3',4'-anhydrovinblastine (AVLB) (15,27,31,34). Most of these processes, although effective, require extremes of reaction temperature and initial activation of the Iboga moiety. In contrast, we report here on a unique, simple reaction for the production of AVLB utilizing ferric ion.

Investigations into reaction conditions resulted in optimal AVLB levels being obtained from the incubation of the monomers with 100mM ferric ion in aqueous acid (0.01N) for a period of 1 hour. Incubation beyond this period, regardless of reaction mixture composition, did not significantly increase AVLB production (c.a. 55% conversion) but rather resulted in the transformation of AVLB to its higher oxidation products, leurosine and catharine (15,31,35-40).

Attempts to decrease oxidative product formation involved incubation of the reaction mixture under an inert atmosphere of argon. This resulted in greater than 77% conversion of monomers to the dimeric product. Similar AVLB levels could also be realized if sparging with argon was conducted immediately prior to reduction of the sample. Addition of L-cysteine or ascorbate (Fig. 1) proved effective under these conditions as well, suggesting a ferroxy complex as being responsible for the observed degradation.

Anhydrovinblastine production was confirmed utilizing a number of physical and spectroscopic techniques. Co-migration of samples with known standard on HPLC and two TLC systems, as well as UV-spectral analysis, provided preliminary evidence for AVLB presence in the incubation mixtures. Further purification by preparative TLC and subsequent mass spectral analysis provided definitive proof for the coupled product as AVLB.

Initial attempts at dimer synthesis (17-21), resulted in the production of compounds exhibiting no biological activity. This was attributed to an inappropriate stereochemistry,

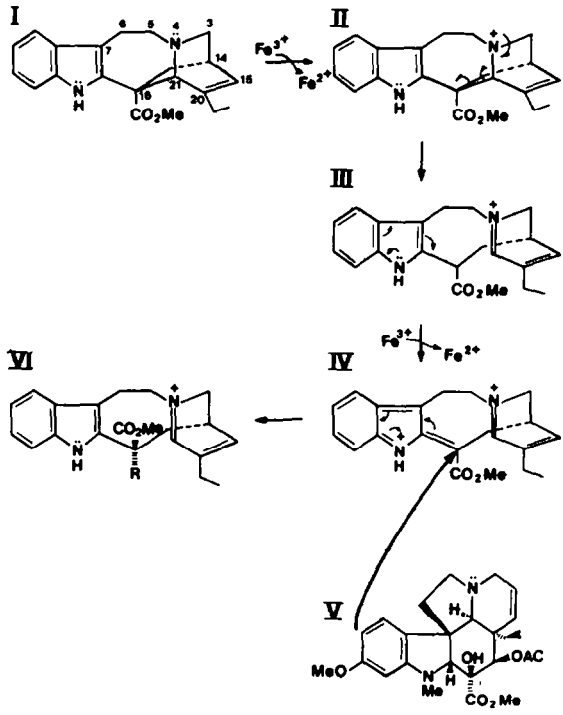

Figure 2: Reaction Mechanism for Iron (III)-Catalysed Dimer Production

A proposed reaction sequence for the coupling of catharanthine and vindoline by Fe(III) is illustrated.

COMPOUND	ION	IONIC RADIUS (um)	IONIZATION POTENTIAL (electron-Volts)	STANDARD POTENTIAL (Volts)	CONVERSION (%)
Ferric chloride	Fe(III)	6.4	30.643	0.770	77
Ferrous Sulfate	Fe(II)	7.4	16.180	0.409	0
Hexachloroplatinic acid	Pt(IV)	6.5	n/a	1.470	0
Rhodium trichloride	Rh(III)	6.8	31.050	0.440	0
Niobium pentachloride	Nb(V)	6.9	50.000	0.344	0
Manganic acetate	Mn(III)	6.6	33.690	1.510	0
Cobalt hexamine chloride	Co(III)	6.3	33.490	1.842	0
Ceric sulfate	Ce(IV)	9.2	36.720	1.700*	0
Molybdic acid	Mo(VI)	6.2	68.000	0.480*	0
Cupric chloride	Cu(II)	7.2	20.292	0.170*	0

*Reference 33
n/a - not available

Table 1: Other Compounds Assessed for Their Ability to Produce AVLB

Compounds capable of generating ions chemically similar to Fe(III) by virtue of their ionic size or oxidizing ability were assessed in the coupling assay. (Data compiled from reference 32 but for (*) which was obtained from reference 33; n/a = not available).

A simpler explanation for the predominance of the alpha coupled product may be that the reaction procedes in a concerted manner (Fig. 3) thereby facilitating vindoline attack via a S_N2 mechanism which would preclude beta coupling (12,35). Such a concerted mechanism can also be explained in terms of the experimental evidence presented. Thus, either of the proposed mechanisms may be valid.

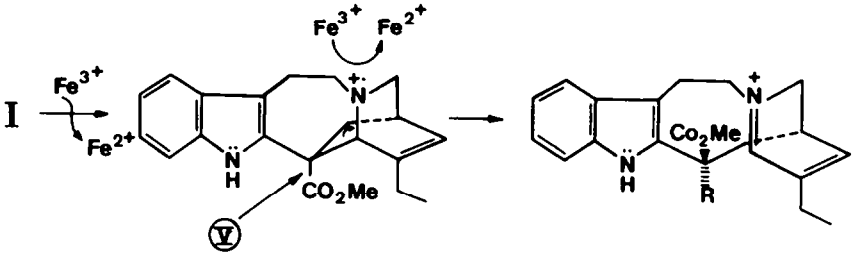

Figure 3: Proposed Mechanism for Concerted Coupling Reaction

Concerted attack of vindoline and subsequent ferric oxidation leads exclusively to α-coupled product.

Whether this reaction is biomimetic is difficult to ascertain but it may provide, in conjunction with recently described enzymatic processes (28-30), an essential precursor for vinblastine and vincristine biosynthesis. This fact is apparently substantiated by the discovery of trace amounts of what appears to be vinblastine amongst the incubation products. However, this evidence is very preliminary and requires further investigation.

In conclusion, a simple economical reaction for the synthesis of 3',4'-anhydrovinblastine from its constitutive monomeric components through ferric ion-mediated coupling in acidic aqueous media has been presented. Levels as high as 77% conversion may be realized if precautions to prevent oxidative degradation are utilized.

EXPERIMENTAL

Catharanthine (CATH-S), as the hydrogen sulfate salt, and vindoline (VIND) were obtained from Gideon Richter (Budapest, Hungary) and were stored dessicated at -20°C until used. AVLB and higher oxidation products (leurosine and catharine) were synthesized as previously described (11). All other chemicals of the appropriate grade were obtained from Fisher Scientific Co. (Toronto, Canada), with the exception of glycine (free base) purchased from Sigma (St. Louis, USA), and utilized without further purification.

The monomer solutions were prepared immediately prior to use in either Milli-Q® grade (Millipore, Mississauga, Canada) water (CATH-S) or 0.01N hydrochloric acid (VIND) and added to the aqueous reaction mixture at a final concentration of 200μM. Coupling was initiated through the addition of ferric or other ions of interest to final concentrations ranging from 20-100mM in a total reaction volume of 6 mL. Aqueous media utilized included 0.1M glycine buffer (pH 2.0, 2.5 and 3.0 at 4°C) and various organic (acetic) and inorganic acids (sulfuric, phosphoric, hydrochloric and nitric).

Samples were incubated, in duplicate, either in air or under an inert argon atmosphere for times of 0.5, 1 and 2 hours with gyratory shaking (ca. 80 cycles/minute) at 4°C. After the designated time period, reactions were quenched by addition of 1 mL of a solution of sodium borohydride, in 14M aqueous ammonium hydroxide, to a final reductant concentration of 10mM. The effect of the addition of anti-oxidants (L-cysteine or ascorbate), on the dimer profile, as opposed to sparging of the sample with high purity argon prior to reduction, was also assessed.

beta-orientation, about the coupling site (9,23,24). Circular dichroism performed on the AVLB sample isolated in the present work, as well as ^{1}H-NMR analysis, confirmed the presence of an alpha-coupled product through corresponding Cotton transitions and the absence of peaks between 4 and 5 ppm in the NMR spectra (9, 23).

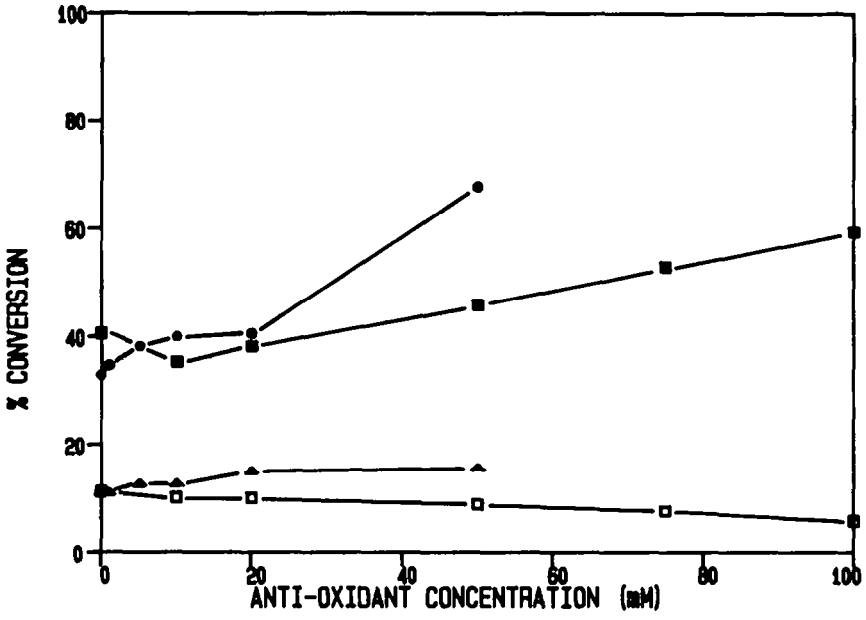

Figure 1: Anti-oxidant Addition and Dimer Formation

Varied levels of the anti-oxidants, L-cysteine and ascorbate were added to the incubation samples immediately prior to reduction with sodium borohydride. Dimer levels achieved (AVLB:L-cysteine(■), oxidized products:L-cysteine(□), AVLB:ascorbate(●) and oxidized products:ascorbate(▲)) are noted above.

Mechanistically, the reaction may be seen to proceed as illustrated (Fig. 2). Ferric catalysed oxidation of the tertiary amine (N_b) of catharanthine (I), as proposed by Smith and co-workers (41-47) leads to the production of the cationic radical species(II). Rearrangement and subsequent fragmentation between C_{16-21} leads to ring opening similar to that achieved with the modified Polonovski reaction (9,10,12,48). Such fragmentation is favoured owing to conjugation of the C_{15-20} double bond and the generated C_{21}-N_b iminium ion as well as the production of a resonance stabilised radical at C_{16}. A second oxidation followed by nucleophilic attack of the diiminium(IV) by vindoline(V) (17,20) results in the formation of the iminium (VI) which upon borohydride reduction yields AVLB (12,28,48,49).

Previous work (12,25,35) has shown that there is an equilibrium established between the two possible diiminium intermediates, prior to coupling, allowing for the production of either alpha (kinetic) or beta (thermodynamic) isomers. Enhancement of kinetic product, utilizing the modified Polonovski reaction, may be achieved at low temperatures by "freezing" the diiminium in the kinetic conformation; however, Iron (III) confers proper stereochemistry at temperatures that favour formation of the thermodynamic product. Therefore, this "freezing" effect may arise as a result of coordination of the ferric ion with the kinetic intermediate thereby accommodating alpha-coupling. This premise appears to be corroborated by the finding that only freely ionizeable ferric sources are capable of supporting the coupling reaction. Ferric complexes including sodium ferric-EDTA, ferritin, ferric pyrophosphate and graphite-immobilized ferric chloride were all found to be inert. The requirement for appropriate co-ordination chemistry as well as redox chemistry is further emphasized by the inability of chemically similar ions (Table 1) to support the coupling reaction.

Alkaloids were recovered through extraction of the samples with ethyl acetate (3 x 6 mL). (Extraction efficiency exceeded 95%). Extracts thus obtained were dried in vacuo, reconstituted in 200µL of HPLC-grade methanol and passed through a 0.45 µM filter (Millipore) immediately prior to HPLC and TLC analysis.

HPLC was performed on a Waters 840 chromatography system (Waters, Mississauga, Canada) utilizing a Pierce 5 µM RP-8 cartridge (14 x 220 mm) (Chromatographic Specialties Inc., Brockville, Canada) in conjunction with a complex gradient (30) of 55-90% methanol in Milli-Q water containing Pic®A modifier (Waters). This regimen provided resolution of all the peaks of interest within a 30 minute run time. Peak identity was verified through UV spectral analysis (190-350 nm) of peaks co-eluting with known standards with a Hewlett-Packard 1040A multiple diode array detector (Hewlett-Packard, Mississauga, Canada).

Further verification of dimer formation was provided through TLC analysis (Silica gel; a) ether:chloroform:methanol (50:35:20) containing 1.5% (v/v) triethylamine) or b) toluene:acetone:methanol:14M ammonium hydroxide (138:49:10:2.5). Sample lanes were scanned on a Shimadzu CS-930 dual wavelength TLC scanner (Tekscience, Oakville, Canada) and the UV spectra generated compared with those of co-migrating standards.

For preparative purposes, pooled samples were developed in TLC solvent system (a). Bands of interest were scraped from the plate and extracted (3 x methylene chloride:methanol (2:1) containing 1.5% (v/v) triethylamine). Alkaloids obtained in this manner were checked for purity by HPLC and subjected to mass spectral and circular dichroism studies, as previously described (30), as well as ^{1}H-NMR ($CDCl_3$) analysis on a Bruker WP80 SY unit.

3', 4'-Anhydrovinblastine (AVLB) ^{1}H-NMR ($CDCl_3$, 500 MHz) δ1.01 (t, J=8Hz, 3H), 2.10(s, 3H), 3.62(s,3H), 3.79(s,3H), 3.82(s,3H), 5.45(s,1H), 5.51(m,1H), 5.84(m, 1H), 6.12(s,1H); HRDEIMS calcd for $C_{61}H_{56}N_4O_8$ 792.4100, obtained 792.4100(±0.2 mmu); CD (EtOH, λ_{max}) 258nm ($\Delta\varepsilon$ +13).

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REFERENCES

1. W.I. Taylor and N.R. Farnsworth (eds) "The Catharanthus Alkaloids, Botany, Chemistry, Pharmacology and Clinical Uses.", Marcel Dekker Inc., New York (1973).

2. W.I. Taylor and N.R. Farnsworth (eds) "The Vinca Alkaloids, Botany, Chemistry and Pharmacology." Marcel Dekker Inc., New York (1973).

3. G.H. Svoboda, I.S. Johnson, M. Gorman and N. Neuss, J. Pharm. Sci. 51, 707 (1962).

4. D.V. Jackson Jr., ISI Atlas of Science: Pharmacology 124 (1987)

5. D.V. Jackson Jr., and R.A. Bender in "Clinical Pharmacology of Anti-Neoplastic Drugs." (Edited by H.M. Pinedo) pp: 277-293. Elsevier/North Holland Biomedical Press, New York (1978).

6. G.H. Svoboda, N. Neuss and M. Gorman, J. Am. Pharm. Assoc. Sci. Ed. 48, 659 (1959).

7. G.C. Cordell in "Kirk-Othmer Encyclopedia of Chemical Technology" (3rd Ed.), Volume 1, pp. 883-943. John Wiley and Sons, New York (1978).

8. M. Lounasmaa and A. Nemes, Tetrahedron 38, 223 (1982).

9. P. Potier, N. Langlois, Y. Langlois and F. Gueritte, J.C.S. Chem. Comm. 670 (1975).

10. J.P. Kutney, A.H. Ratcliffe, A.M. Treasurywala and S. Wunderly, Heterocycles 3, 639 (1975).

11. N. Langlois and P. Potier, Tetrahedron Lett. 1099 (1976).

12. J.P. Kutney, T. Hibino, E. Jahngen, T. Okutani, A.H. Ratcliffe, A.M. Treasurywala and S. Wunderly, Helv. Chimica Acta 59, 2858 (1976).

13. P. Potier, Chimia 30, 544 (1976).

14. S. Raucher, B.L. Bray and R.F. Lawrence, J. Am. Chem. Soc. 109, 442 (1987).

15. A.I. Scott, F. Gueritte and S.-L. Lee, J. Am. Chem. Soc. 100, 6253 (1978).

16. N. Langlois and P. Potier, J.C.S. Chem. Comm. 582 (1979).

17. G. Büchi, R.E. Manning and J.A. Monti, J. Am. Chem. Soc. 86, 4531 (1964).

18. J. Harley-Mason and Attu-ur-Rahman, Chem. Commun. 1048 (1967).

19. W.I. Taylor, Proc. Chem. Soc. 247 (1962).

20. G. Büchi and R.E. Manning, J. Am. Chem. Soc. 88, 2532 (1966).

21. J.P. Kutney, J. Beek, F. Bylsma and W.J. Cretney, J. Am. Chem. Soc. 90, 4504 (1968).

22. A.U. Rahman, A. Basha and M. Ghazala, Tetrahedron Lett. 2351 (1976).

23. J.P. Kutney, D.E. Gregonis, R. Imhof, I. Itoh, E. Jahnger, A.I. Scott and W.K. Chan, J. Am,. Chem. Soc. 97, 5013 (1975).

24. P. Potier in "Stereoselective Synthesis of Natural Products." Proceedings of the Seventh Workshop Conference Hoechst, 24-27 Sept., 1978, pp. 19-27. Excerpta Medica, Amsterdam (1979).

25. P. Potier, J. Nat. Prod. 43, 72 (1980).

26. M. Gorman, N. Neuss and N.J. Cone, J. Am. Chem. Soc. 87, 93 (1965).

27. R.L. Baxter, C.A. Dorschel, S.-L. Lee and A.I. Scott, J.C.S. Chem. Comm. 257 (1979).

28. M. Misawa, T. Endo, A.E. Goodbody, J. Vukovic, C. Chapple, L. Choi and J.P. Kutney, Phytochemistry (submitted).

29. T. Endo, A.E. Goodbody, J. Vukovic and M. Misawa, Phytochemistry (submitted).

30. A.E. Goodbody, T. Endo, J. Vukovic, J.P. Kutney and M. Misawa, Planta Medica (submitted).

31. N. Langlois and P. Potier, J.C.S. Chem. Comm. 102 (1978).

32. R.C. Weast (ed), "CRC Handbook of Chemistry and Physics" (58th Ed.), CRC Press Inc., Palm Beach, Fla. (1978).

33. J.A. Dean (ed), "Lange's Handbook of Chemistry", (12th Ed.), McGraw-Hill Book Co., New York (1979).

34. K.L. Stuart, J.P. Kutney, T. Honda and B.R. Worth, Heterocycles 9, 1391 (1978).

35. J.P. Kutney, A.H. Ratcliffe, A.M. Treasurywala and S. Wunderly, Heterocycles 3, 639 (1975).

36. J.P. Kutney, J. Balsevich, G.H. Bokelman, T. Hibino, I. Itoh and A.H. Ratcliffe, Heterocycles 4, 997 (1976).

37. J.P. Kutney, J. Balsevich and B.R. Worth, Heterocycles 9, 493 (1978).

38. K.L. Stuart, J.P. Kutney and B.R. Worth, Heterocycles 9, 1015 (1978).

39. J.P. Kutney, J. Balsevich and B.R. Worth, Can. J. Chem. 57, 1682 (1979).

40. J.P. Kutney, B. Aweryn, L.S.L. Choi, P. Kolodziejczyk, W.G.W. Kurz, K.B. Chatson and F. Constabel, Helv. Chimica Acta 65, 1271 (1982).

41. J.R. Lindsay Smith, R.O.C. Norman and W.M. Walker, J. Chem. Soc. (B), 269 (1968).

42. C.A. Audeh and J.R. Lindsay Smith, J. Chem. Soc. (B), 1280 (1970).

43. C.A. Audeh and J.R. Lindsay Smith, J. Chem. Soc. (B), 1741 (1971).

44. C.A. Audeh and J.R. Lindsay Smith, J. Chem. Soc. (B), 1745 (1971).

45. P.A. Bather, J.R. Lindsay Smith and R.O.C. Norman, J. Chem. Soc. (B), 3060 (1971).

46. J.R. Lindsay Smith, R.O.C. Norman and A.G. Rowley, J. Chem. Soc. Perkin. Trans I, 228 (1972).

47. J.R. Lindsay Smith and L.A.V. Mead, J. Chem Soc. Perkin Trans II, 206 (1973).

48. N. Langlois, F. Gueritte, Y. Langlois and P. Potier, J. Am. Chem. Soc. 98, 7017 (1976).

49. J.P. Kutney, Heterocycles 25, 617 (1984).