study,[5] and was converted with triethylamine[1] to the $B_{10}H_6D_4^{-2}$ ion. The B^{11} n.m.r. spectrum of the latter was compared with that of $(Et_3NH)_2$-$B_{10}H_{10}$ in acetonitrile

	δ (in p.p.m. ± 0.5 relative to $B(OCH_3)_3$)	J_{BH} (in c./s. ± 5)
$(Et_3NH)_2B_{10}H_{10}$ axial	18.8	138
$(Et_3NH)_2B_{10}H_{10}$ equatorial	47.0	125
$(Et_3NH)_2B_{10}H_6D_4$ axial	19.0	143
$(Et_3NH)_2B_{10}H_6D_4$ equatorial	48.1	Broad singlet

No change was apparent in the low field doublet ascribed to apical BH units, while appreciable collapse was observed in the high field doublet attributed to the equatorial belt of eight equivalent BH units. Because of the inherent line widths of B^{11} resonances, line shapes are only moderately sensitive indices of deuteration. At present, we can conclude that at least 90% of the apical positions of $B_{10}H_6D_4^{-2}$ contained BH bonds, whereas random distribution of deuteriums would predict 60%. Thus, the available data are consistent with the proposed structure[2] and the mechanism of ion formation outlined here.

It might further be mentioned that infrared studies show $B_{10}H_{10}^{-2}$ does not undergo deuterium exchange with D_2O or $(Et_3ND)^+$.

(5) J. A. Dupont and M. F. Hawthorne, Meeting Abstracts, p. 47-N, 138th Meeting of the American Chemical Society, New York, New York, September 11–16, 1960.

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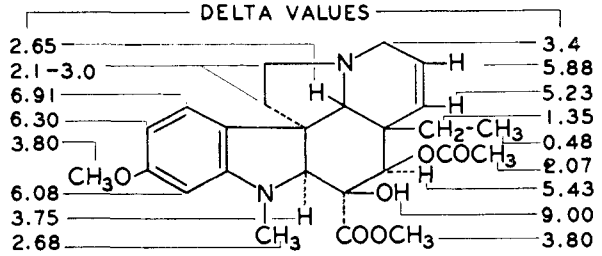
VINCA ALKALOIDS. X[1]. THE STRUCTURE OF VINDOLINE

Sir:

Vindoline,[2,3] $C_{25}H_{32}O_6N_2$, is the major alkaloid in the leaves of *Vinca rosea* Linn. It occurs not only as the free base which possesses little biological activity, but combined with various indolic moieties[4] it is ubiquitous in the dimeric *Vinca* alkaloids such as vinblastine[5] (VLB) and leurocristine,[1] which are potent oncolytic agents.[6]



I

(1) Paper IX, G. H. Svoboda, *Lloydia*, **24**, 173 (1961).

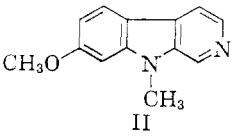
(2) M. Gorman, N. Neuss, G. H. Svoboda, A. J. Barnes, Jr., and N. J. Cone, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 256 (1959).

(3) G. H. Svoboda, N. Neuss, and M. Gorman, *ibid.*, **48**, 659 (1959).

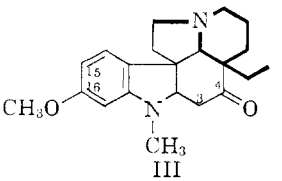
(4) M. Gorman, N. Neuss, and G. H. Svoboda, *J. Am. Chem. Soc.*, **81**, 4745 (1959).

(5) Previously called vincaleukoblastne: N. Neuss, M. Gorman, G. H. Svoboda, G. M. Maciak, and C. T. Beer, *ibid.*, **81**, 4754 (1959).

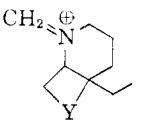
The base was previously shown[2] to be pentacyclic and to contain an isolated double bond. Five of the oxygens were found to be present as hydroxyl, carbomethoxyl and acetoxyl functions.



II



III



IV, Y = C=O
V, Y = CH_2

Structure I has been found to be consonant with all observed spectral and chemical data for vindoline. The stereochemical implications are quite tentative and rest on evidence which will be presented in the full paper. Examination of the n.m.r. spectrum[7] of vindoline allows the assignment of each proton as shown. The assignments, however, deserve some comment. The unsplit peak of the proton on the carbon bearing the acetoxyl moiety shifts to 4.07δ in desacetylvindoline. The protons of the methylene portion of the C-ethyl group are not equivalent and are found as a 12-band multiplet centered at 1.35δ (J = 7.5 c.p.s.). The methyl triplet, however, is almost symmetrical in vindoline, being found at an exceptionally high field (0.48δ, J = 7.5 c.p.s.) for a grouping of this type. This high field appears to be the result of increased shielding by ring currents from the aromatic ring. The coupling constant of the two *cis* vinyl protons is J = 10 c.p.s., and one of these (5.88δ) is further split by two non-equivalent adjacent protons (δ = 3.4) with coupling constants J = 5 and 2 c.p.s.

The position of the aromatic methoxyl is shown to be at either C-15 or C-16[8] by examination of the typical 1,2,4 aromatic proton pattern (*ortho* splitting J = 8 c.p.s., *meta* splitting J = 2.5 c.p.s.). The final selection of C-16 is based on the comparison of the infrared and ultraviolet spectra of vindoline with 6-methoxy-N-methyldihydroindole[9] and by the isolation of *ind*-N-methylnorharmine (II); m.p. 112–114°, hydrochloride m.p. 232–236°, molecular weight (M) 212 (by mass spectrometry), λ_{max}^{EtOH} 243 (37,500), 303 (15,100), 336 (4,300), from a soda lime distillation of vindoline at 325°. This derivative also indicates the position of the N-CH_3 as being on the anilino nitrogen.[10] A

(6) I. S. Johnson, H. F. Wright, and G. H. Svoboda, *Abstract of the Proc. Am. Assoc. for Cancer Research*, Vol. 3, No. 4 (1962) (in press) and references cited therein.

(7) Spectra recorded on Varian HR-60 in $CDCl_3$ with tetramethylsilane as internal standard: Values δ(PPM), TMS = 0.

(8) For numbering system see C. Djerassi, *et al.*, *Proc. Natl. Acad. Sci. U.S.*, **48**, 113 (1962).

(9) Kindly supplied by Dr. A. Hofmann, Sandoz A. G., Basel, Switzerland.

(10) The presence of the dimethylene "tryptophan" bridge is also strongly supported by the isolation of *ind*-N-methylnorharmine and the finding that tryptophan is an excellent *in vivo* precursor for vindoline (private communication, R. McMahon and M. Gorman, Eli Lilly and Company).

second product (III) was isolated from the above reaction but could be obtained in better yield as described below.

Hydrogenation of vindoline (PtO_2/EtOH–HCl) afforded dihydrovindoline, $C_{25}H_{34}O_6N_2$, (m.p. 121–124° or 164–166°, pK_a' 5.9, no vinyl protons but four new protons at 1.5δ in the n.m.r., $[\alpha]^{25}D$ +52.4° [C = 1, $CHCl_3$]) which could be converted to an amorphous hygroscopic hydrochloride. Pyrolysis of this salt at 195–200° in vacuum gave a distillate from which the above second "soda lime" compound was obtained by direct crystallization from hexane in 15% over-all yield, $C_{21}H_{28}O_2N_2$, m.p. 130–132°, pK_a' 5.35, $[\alpha]^{25}D$ +12° (C = 1, $CHCl_3$), mol. wt. 340. The methoxydihydroindole portion of this compound was the same as in vindoline and the second oxygen was found to be present as a ketonic carbonyl ($\lambda_{max.}^{CHCl_3}$ 5.85 μ).

Careful comparison of the mass spectra of the pyrolysis ketone (III) and dihydrovindoline with that of N-methyl desacetyl aspidospermine[11] indicated the presence of the latter ring system in vindoline and its derivatives, since in all three compounds intense peaks were found at *m/e* 124, 174, 188 and 298.

The position of the carbonyl in III was assigned on the basis of the presence of the peak at *m/e* 298[11] (= M-42) which suggested that this group involves either C-3 or C-4. Position 4 was selected for two reasons: the typical ABX pattern at low field (3 to 3.5δ) in the n.m.r. spectrum (also consistent with structure III) indicated three protons between the carbonyl group and nitrogen; and equilibration with CH_3OD/methoxide resulted in the introduction of only two deuterium atoms per molecule (mol. wt., 342). The peak at *m/e* 124 comprises the atoms outlined by heavy lines in structure III while the 174 and 188 peaks contain the dihydroindole moiety (as indoles) with one or two carbons, respectively, of the "tryptophan" dimethylene bridge attached. The fragment of mass 298 involves the loss of the two-carbon bridge (C-3 and C-4) containing the oxygen functions (except the aromatic methoxyl) of each derivative. The only other major peak in the ketone occurs at *m/e* 166 which shifts to *m/e* 168 in the deuterio derivative. Structure IV is assigned to it since this peak corresponds to fragment V of mass 152[12] in the spectrum of aspidospermine.[13]

The mass spectral evidence, therefore, besides indicating the ring system of vindoline shows that the oxygen functions other than the aromatic methoxyl can only be present at positions 3 and 4, and therefore must be arranged as shown in structure I to be consistent with n.m.r. data. The formation of the ketone is also explained easily from this formulation (dehydration, hydrolysis, decarboxylation).

It is interesting to note that vindolinine,[8] another *Vinca* alkaloid with a slightly modified aspidospermine skeleton, contains the double bond at the same position[8] as vindoline.

We gratefully acknowledge helpful discussions with Dr. J. N. Shoolery, Varian Associates; Professor Carl Djerassi, Stanford University; Professor J. C. Martin, University of Illinois; and Dr. Harold Boaz, Eli Lilly and Company. Thanks also are due to Dr. Boaz's associates for physical data.

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RECEIVED JANUARY 19, 1962

(11) K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *Tetrahedron Letters*, No. 14, 485 (1961).

(12) K. Biemann, Symposium on Mass Spectrometry, Oxford, September, 1961.

(13) The fragmentation is more complex in dihydrovindoline, giving rise to a series of peaks at *m/e* 398, 311, 284 and 224 which are consistent with the proposed structure but will be discussed in the full paper.

THE BIOSYNTHETIC INCORPORATION OF DOPAMINE INTO HYDRASTINE[1]

Sir:

Recently we showed[2] that hydrastine (III) is derived from two tyrosine units, which, however, are not incorporated with equal efficiency. This was the first demonstration of differential utilization of a single precursor in the biosynthesis of the two segments of a "dimeric" alkaloid.[3]

This differential utilization of tyrosine implies that the "doubling" step of the anabolic sequence is preceded by one or more dissimilar structural modifications of either or both tyrosine units. It is likely that tyrosine gives rise by independent pathways to two different "monomeric" metabolic intermediates which join to form a "dimeric" precursor from which the alkaloid is then derived.

We have now found that 3,4-dihydroxyphenylethylamine (dopamine) can serve as a precursor of one, but not of the other, of these "monomeric" intermediates.

α-^{14}C-Dopamine hydrobromide (II) was infused by means of cotton wicks into the stems of six three-year old plants of *Hydrastis canadensis* L. In a simultaneous separate experiment, DL-β-^{14}C-tyrosine (I) was administered similarly to four three-year old plants. After nine days the plants were harvested and the alkaloids extracted from the roots. Chemical and radioactive yields are recorded in Table I.

Degradation of hydrastine was carried out as previously reported.[2] The activities of radiodopamine-derived hydrastine and its degradation products (Table II) clearly demonstrate that radiocarbon was confined to carbon atom 3, and show that dopamine serves as a specific precursor of hydrastine, *but that only one dopamine unit is utilized in the biosynthesis of the alkaloid.* Hydrastine isolated from plants fed with radiotyrosine, on the other hand, was derived from two tyrosine units.[4]

(1) Financial support by the National Research Council of Canada and by the Ontario Research Foundation is gratefully acknowledged.

(2) J. R. Gear and I. D. Spenser, 140th A.C.S. Meeting, Chicago, September, 1961, Abstracts; *Nature* (London) **191**, 1393 (1961).

(3) Similar results have since been obtained in a study of the biosynthesis of morphine from carbon-14-dioxide (H. Rapoport, N. Levy and F. R. Stermitz, *J. Am. Chem. Soc.*, **83**, 4298 (1961).

(4) Since specific incorporation of radioactivity from α-^{14}C-tyrosine into carbon atoms 1 and 3 of the hydrastine molecule already has been demonstrated,[3] it was sufficient in the present case to separate the two labelled carbon atoms (4 and 7'), derived from β-^{14}C-tyrosine, from one another, and unnecessary to isolate them completely.