malities that would be anticipated from such serious eclipsing.

Current efforts are directed toward the elucidation of the detailed genesis of VII. These results and a full description of all products formed in the dehydroiodination of IV will be described in detail in a subsequent publication.

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RECEIVED FEBRUARY 15, 1962

VINCA ALKALOIDS. XI.[1] STRUCTURES OF LEUROCRISTINE (LCR) AND VINCALEUKOBLASTINE (VLB)[2]

Sir:

The unique biological properties of leurosine and vincaleukoblastine (VLB) have been thoroughly reviewed.[3] The latter alkaloid has been introduced clinically for the treatment of Hodgkins' disease and choriocarcinoma.[4] Recently Svoboda described another alkaloid from *Vinca rosea* Linn.[5] called leurocristine (LCR) which appears to possess a different spectrum of oncolytic activity in experimental and human neoplasms.[6,7]

It is the purpose of this communication to demonstrate that LCR[5] is des-N(a)-methyl-N(a)-formyl-VLB (N(a) being the anilino-nitrogen in the vindoline moiety of the molecule), and that these compounds represent the first examples of indole-indoline alkaloids in which the indole moiety is linked through a C–C bond to the aromatic ring of the dihydroindole portion of the molecule.

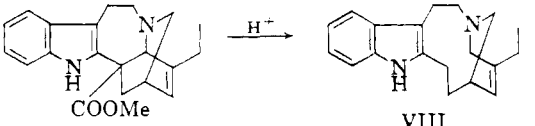
The n.m.r. spectrum of VLB, $C_{46}H_{56}O_9N_4$ (I),[8] shows these functional groups with corresponding chemical shifts: $COOCH_3$ and aromatic OCH_3 both at 3.8δ, $COOCH_3$ 3.63δ, N-CH_3 2.73δ, $OCOCH_3$ 2.12δ and NH(indole) 8.09δ. The remaining two oxygens are present as hydroxyls (free and hydrogen bonded). Their presence is easily detected in the infrared spectrum and has been substantiated by the preparation of a diacetate (ketene in benzene), $C_{50}H_{60}O_{11}N_4$[9] (II), 168–170° (dec.), $[\alpha]^{25}D$ −26.4° ($CHCl_3$). Accordingly, the n.m.r. spectrum of (II) shows three acetyl methyl peaks at 1.98, 2.09 and 2.40δ. Its comparison with the n.m.r. spectrum of VLB base indicates that the free hydroxyl (*vide supra*) is tertiary. This is apparent from the absence of a new signal in the range of 3.9–5.5δ (except for the known C-2 proton which shifts from 3.75 to 4.09δ[1], Fig. 1).

Analyses[8] of LCR base (III), sulfate and monomethiodide are consistent with a formulation of $C_{46}H_{54}O_{10}N_4$. Its ultraviolet spectrum, λ_{max}^{EtOH} 220 mμ (log a_M 4.65), 255 mμ (log a_M 4.21), 296 mμ (log a_M 4.18) and λ_{min}^{EtOH} 275 mμ (log a_M 4.02) is quite different from that of VLB and indicates a different substitution on N(a) of the dihydroindole moiety.

The infrared spectra of VLB and LCR are quite similar with the exception of the presence of a strong additional band, $\lambda_{max}^{CHCl_3}$ 5.94 μ in the spectrum of the latter. The n.m.r. spectra differ in that the N-CH_3 proton resonance at 2.73δ present in VLB is missing in LCR; and, conversely, in place of only one low field, 9.8δ proton in VLB, there are two in LCR at 9.5 and 8.9δ.

Lithium aluminum hydride reduction of the two alkaloids afforded good yields of the same[9] pentahydroxy derivative, $C_{42}H_{54}O_6N_4$[10] (IV), pK_a' 5.34 and 8.2 (33% DMF), m.p. 213–215° (dec.), $[\alpha]^{26}D$ −117.2° ($CHCl_3$). This formulation is consistent with the reduction of two methyl esters, one acetate and the N-formyl group in the case of LCR. The n.m.r. spectrum of IV shows accordingly only two methyl signals, aromatic OCH_3 at 3.8δ and N-CH_3 at 2.73δ. The position of N-CH_3 in VLB or N-CHO in LCR as well as other functional groups was demonstrated from the products of acid cleavage (concd. hydrochloric acid, $SnCl_2$, tin-metal, reflux) carried out on these alkaloids and leurosine. In each case there was obtained upon chromatography of the reaction mixture an indole compound (*vide infra*) followed by vindoline derivatives. VLB and leurosine afforded desacetylvindoline (V),[10,11] thus proving the identity of the dihydroindole portion of these alkaloids. The corresponding fraction from the cleavage of LCR yielded des-N(a)-methyldesacetylvindoline (VI), M = 400, $C_{22}H_{28}O_5N_2$. The mass spectrum as well as ultraviolet and infrared spectra demonstrate the relationship of this compound to desacetylvindoline. The structure of vindoline has been established recently,[1] and since both VLB and LCR yielded the same[10] tetracyclic indole derivative, velbanamine (VII), $C_{19}H_{26}ON_2$, m.p. 139–141°, pK_a' 8.8, $[\alpha]^{25}D$ +56.2° ($CHCl_3$), M = 298, the two alkaloids differ only in the manner mentioned above.

A related tetracyclic indole derivative, cleavamine (VIII); $C_{19}H_{24}N_2$, M = 280, m.p. 109–113°, $[\alpha]^{26}D$ +68° ($CHCl_3$), pK_a' 8.2 (33% DMF) was obtained from the cleavage of leurosine.[12] Isolation



IX VIII

(1) Vinca. X: M. Gorman, N. Neuss and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962).

(2) A.M.A.-approved generic names are vincristine and vinblastine, respectively. VLB is supplied as VELBAN® (vinblastine sulfate, Lilly).

(3) I. S. Johnson, Howard F. Wright, Gordon H. Svoboda and Janet Vlantis, *Cancer Research*, **20**, 1016 (1960).

(4) (a) M. E. Hodes, R. J. Rohn and W. H. Bond, *Canadian Cancer Conference*, **4**, 373 (1962); (b) O. H. Warwick, J. M. M. Darte and J. S. Olin, *ibid.*, 373; (c) Roy Hertz, *ibid.*, 399.

(5) G. H. Svoboda, *Lloydia*, **24**, 173 (1961).

(6) I. S. Johnson, H. F. Wright and G. H. Svoboda, *Proc. Am. Assn. for Cancer Research*, **3** (4) in press (1962).

(7) *Inter al*, J. G. Armstrong, R. W. Dyke and P. J. Fouts, *ibid.*, **3** (4), 1962 (In press).

(8) N. Neuss, M. Gorman, G. H. Svoboda, G. M. Maciak and C. T. Beer, *J. Am. Chem. Soc.*, **81**, 4754 (1959). In view of the new chemical evidence we prefer the present formula over $C_{46}H_{58}O_9N_4$ reported therein. Satisfactory analyses were obtained on all compounds for which empirical formulas are given.

(9) Identity was established by the comparison of m.p., X-ray powder patterns and infrared spectra in chloroform solution.

(10) N. Neuss, M. Gorman and G. H. Svoboda, Plant Chemists Meeting, Columbia University, February 12, 1960, New York, N. Y.

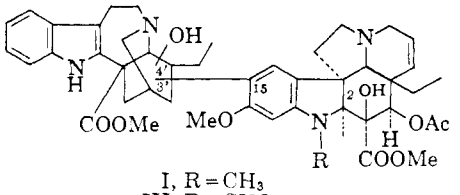
(11) Desacetylvindoline is obtained from vindoline by a mild hydrolysis, M. Gorman, N. Neuss, G. H. Svoboda, A. J. Barnes and N. J. Cone, *J. Am. Pharm. Assn. Sci. Ed.*, **48**, 256 (1959).

(12) The correct formula of leurosine is still in doubt because of difficulty of preparing a solvent free sample.

of cleavamine demonstrates the relationship of the indole portion of C_{46} alkaloids to catharanthine[13] (IX) since it was also obtained by a similar acid treatment of the latter.[14]

The mode of attachment of the indole moiety to the vindoline portion of VLB and LCR is apparent from the n.m.r. spectra of these compounds. The aromatic protons of the indole portion are unchanged; however, instead of the typical 1,2,4 pattern (6.9, 6.3, 6.08δ) present in vindoline,[1] the spectrum contains only two protons (δ6.16, 6.70) in a 1,4 relationship. A similar resonance pattern occurs also in vindoline derivatives substituted in the para position to the N(a) (*e.g.*, bromovindoline). *Therefore, the missing proton at C-15 (Fig. 1) represents the site of attachment of the indole portion.*

These data clearly indicate the partial structures I and III for VLB and LCR, respectively. On the basis of biogenetic considerations (free radical photosynthesis), as well as the mode of cleavage of these alkaloids, we are prompted to propose either C-3′ or C-4′ as the most likely site of attachment in the indole moiety and possible positions of the tertiary hydroxyl. The elucidation of this problem is at present in progress in these laboratories.



I, R = CH_3
III, R = CHO

We gratefully acknowledge helpful discussions with Prof. J. C. Martin, University of Illinois. Thanks are due to Dr. G. H. Svoboda for the sample of leurocristine, to Prof. K. Biemann, Massachusetts Institute of Technology, for mass spectra and their interpretation, and to Dr. J. N. Shoolery, Varian Associates, for n.m.r. spectra.

(13) M. Gorman, N. Neuss and G. H. Svoboda, *J. Am. Chem. Soc.*, **81**, 4745 (1959), and N. Neuss and M. Gorman, *Tetrahedron Letters*, No. 6, 206 (1961).

(14) Cleavamine is believed to possess structure VIII and velbanamine appears to be its dihydrohydroxy derivative. The mass spectra indicate the same carbon skeleton for the two compounds and are consistent with the proposed structures. The rearrangements of catharanthine and related compounds under acidic conditions will be reported elsewhere, M. Gorman and N. Neuss, *Tetrahedron*, 1962 (in preparation).

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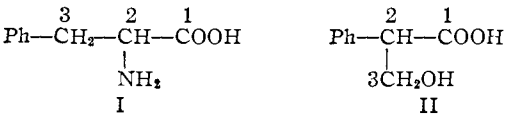
THE BIOSYNTHESIS OF TROPIC ACID[1]

Sir:

We have established[2,3] recently by means of tracer experiments that phenylalanine (I) is a precursor of tropic acid (II), the acid moiety of the ester alkaloids hyoscyamine and hyoscine. In particular it was shown that C_2 and C_3 of the side chain of tropic acid were derived from C_3 and C_2, respectively, of the phenylalanine. The origin



of the carboxyl group remained in doubt. Goodeve and Ramstad[4] obtained carboxyl-labeled tropic acid when tryptophan-3-C^{14} was fed to *Datura stramonium* plants. We attempted to explain this rather surprising result by suggesting[3] that the radioactive tryptophan was metabolized in the plant yielding radioactive carbon dioxide which was then incorporated into tropic acid by a hypothetical carboxylation of phenylpyruvic acid or related compound.

We also considered that the branched three-carbon side chain of tropic acid might arise by a rearrangement of the phenylalanine side chain. This hypothesis was tested by feeding L-phenylalanine-1-C^{14}[5] (1.21 mg., 50 μc.) to ten two-month old *Datura stramonium* plants[6] growing in soil using a wick arrangement.[7] After two weeks hyoscyamine and hyoscine were isolated without dilution by established methods[2] and had specific activities of 3.6×10^6 and 3.0×10^6 d.p.m./mM.,[8] respectively. Hydrolysis of the radioactive hyoscyamine yielded inactive tropine and tropic acid (3.6×10^6 d.p.m./mM.). Dehydration of the tropic acid with 50% aqueous potassium hydroxide[9] afforded atropic acid which was oxidized in alkaline solution with sodium metaperiodate and a catalytic amount of osmium tetroxide yielding formaldehyde collected as its dimedone derivative (inactive) and phenylglyoxylic acid isolated as its oxime (3.4×10^6 d.p.m./mM.)[10]. The oximino acid was refluxed in water when decarboxylation occurred yielding benzonitrile.[11] The evolved carbon dioxide was collected as barium carbonate (3.5×10^6 d.p.m./mM.). The benzonitrile was hydrolyzed yielding inactive benzoic acid. Benzoic acid obtained by the direct oxidation of the radioactive tropic acid was also completely inactive. This degradation thus established that the tropic acid was labeled solely on its carboxyl group. The relatively high incorporation[12] (0.25%) of tracer into the tropic acid, with specific labeling, favors the hypothesis that the side chain of tropic acid is indeed produced by a novel intramolecular

(1) This investigation was supported by a Research Grant MY-2662, from the National Institute of Mental Health, U. S. Public Health Service.

(2) E. Leete, *J. Am. Chem. Soc.*, **82**, 612 (1960).

(3) E. Leete and M. L. Louden, *Chemistry and Industry*, 1405 (1961).

(4) A. M. Goodeve and E. Ramstad, *Experientia*, **15**, 124 (1961).

(5) Purchased from Nichem Ind., Bethesda, Maryland.

(6) We thank Robert C. McLeester of the Botany Department of the University of Minnesota for the cultivation of the *Datura* plants.

(7) C. L. Comar, "Radioisotopes in Biology and Agriculture," McGraw-Hill Book Co., Inc., New York, N. Y., 1955, p. 151.

(8) Radioactivity measurements were carried out in a Nuclear Chicago Model C-115 low background Q gas flow counter. Determinations were carried out on samples of finite thickness, making corrections for efficiency and self absorption.

(9) J. W. Baker and A. Eccles, *J. Chem. Soc.*, 2125 (1927).

(10) This slightly low value may be due to contamination with benzonitrile which is formed in small amount on sublimation of the oxime.[11]

(11) A. Ahmad and I. D. Spenser, *Can. J. Chem.*, **39**, 1340 (1961).

(12) Incorporation is defined as the total amount of radioactivity found in the isolated natural product divided by the total amount of tracer fed to the plant.