ture of this compound. Treatment of commercial caryophyllene with sulfuric acid in ether produced variable, but always small, amounts of the crystalline alcohol together with clovene and β-caryophyllene alcohol. Since commercial caryophyllene contains up to 10% of humulene, the reported acid-catalyzed conversion[2] of humulene to a crystalline alcohol seemed to us significant.

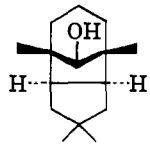
G.l.c. pure caryophyllene on treatment with concentrated sulfuric acid in ether produced no detectable amount of the α-alcohol, whereas both α- and β-humulene[3] were converted to the required alcohol in approximately 40% yield. The physical constants of this compound and its derivatives were in agreement with those reported by Nickon.[4]

The α-alcohol readily formed a *p*-bromobenzenesulfonate which was obtained from ether solution as monoclinic crystals, m.p. 147.5–148°, with cell dimensions $a = 12.65$, $b = 10.82$, $c = 16.43$ Å., $\beta = 109°13'$. There are four molecules of $C_{21}H_{29}O_3BrS$ in the unit cell and the space group is $P2_1/c$. Three-dimensional X-ray intensity data were recorded on equi-inclination Weissenberg photographs and estimated visually; in all 2100 F_o values were derived.

The coordinates of the bromine and sulfur atoms were obtained from Patterson syntheses and the remaining atoms, apart from hydrogen, were then located by evaluating successive three-dimensional electron-density distributions. Further refinement of the atomic coordinates is being continued by the method of least squares; the value of R is now 16%.

The results of the crystallographic analysis unambiguously define the constitution and stereochemistry of the alcohol to be as in I. The cyclohexane ring has the usual chair conformation with the hydroxyl group axial.

The average valency angle in the six-membered ring is 109°. In the five-membered rings, however, the average valency angle is 104°, distinctly smaller than tetrahedral and in good agreement with values reported for five-membered rings in other molecules.[5]



I

Acknowledgment.—The calculations were performed on the Glasgow University Deuce computer with programs[6] devised by Dr. J. S. Rollett and Dr. J. G. Sime. We are grateful to Prof. J. Monteath Robertson, F.R.S., and Prof. R. A. Raphael, F.R.S., for their constant interest and encouragement. We thank D.S.I.R. for a studentship (J. S. R.) and the Carnegie Trust for a scholarship (K. W. G.).

CHEMISTRY DEPARTMENT
THE UNIVERSITY
GLASGOW, W. 2
SCOTLAND

K. W. GEMMELL
W. PARKER
J. S. ROBERTS
G. A. SIM

RECEIVED FEBRUARY 1, 1964

(2) S. Dev, *Current Sci.* (India), **20**, 296 (1951).

(3) V. Benešová, V. Herout, and F. Šorm, *Collection Czech. Chem. Commun.*, **26**, 1832 (1961). We are indebted to Dr. V. Herout for a sample of humulene.

(4) A. Nickon, J. R. Mahajan, and F. J. McGuire, *J. Org. Chem.*, **26**, 3617 (1961). Workers at Johns Hopkins University have independently arrived at the same structure by an elegant degradation sequence, and simultaneous publication was arranged [A. Nickon, F. J. McGuire, J. R. Mahajan, B. Umezawa, and S. A. Narang, *J. Am. Chem. Soc.*, **86**, 1437 (1964)].

(5) See, *inter alia*, I. C. Paul, G. A. Sim, T. A. Hamor, and J. M. Robertson, *J. Chem. Soc.*, 4133 (1962); J. A. Hamilton, T. A. Hamor, J. M. Robertson, and G. A. Sim, *ibid.*, 5061 (1962); J. Donohue and K. Trueblood, *Acta Cryst.*, **5**, 419 (1952).

(6) "Computing Methods and the Phase Problem in X-ray Crystal Analysis," R. Pepinsky, J. M. Robertson, and J. C. Speakman, Ed., Pergamon Press, Oxford, 1961; J. S. Rollett, p. 87; J. G. Sime, p. 301.

High Resolution Mass Spectra of Natural Products.[1,2] Vinblastine[3] and Derivatives

Sir:

We wish to present an example illustrating the usefulness of high resolution mass spectrometry[4] for the determination of the structure of a molecule as complex as the "dimeric" indole alkaloid vinblastine (VLB)[5] where this technique was used to establish the correct elemental composition,[6] to provide completely independent additional information regarding the point of attachment of the two parts, and to corroborate other features of the molecule.

Conventional mass spectra of VLB indicated a molecular weight 838 which was incompatible with the earlier structure.[7] However, high resolution mass spectrometry, employed in a fashion[8] that permits detecting any thermal changes taking place in the sample,[10] showed that vinblastine is, in fact, thermally labile. When it begins to vaporize, mass 810 predominates, but peaks at $m/e = 824$ and even $m/e = 838$ appear immediately and increase with time. The determined mass of these species (Table I) differ, in fact, by CH_2, ruling out any nonhomologous contaminants. Fractionation of contaminants is eliminated by the fact that under conditions favoring thermal reactions (slow

(1) Application of Mass Spectrometry to Structure Problems. Part XIX[2]; this investigation was supported by grants from the National Science Foundation (G-21037) and the National Institutes of Health, Public Health Service (GM-09352).

(2) Part XVIII: W. Benz and K. Biemann, *J. Am. Chem. Soc.*, in press.

(3) This A.M.A. approved generic name refers to the alkaloid vincaleukoblastine.

(4) For reviews of previous work in this field using a different technique (see ref. 8 below), see J. H. Beynon, "Advances in Mass Spectrometry," Vol. II, R. M. Elliot, Ed., Pergamon Press, Oxford, 1963, pp. 216–229; and R. A. Saunders and A. E. Williams, "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press, New York, N. Y., 1963, pp. 343–396, and references therein.

(5) N. Neuss, *et al.*, *J. Am. Chem. Soc.*, **86**, 1440 (1964).

(6) Neither the usual analytical data (see footnote 9 in ref. 5) nor conventional mass spectrometric data (discussed below) gave unambiguous results.

(7) N. Neuss, M. Gorman, H. E. Boaz, and N. J. Cone, *J. Am. Chem. Soc.*, **84**, 1509 (1962).

(8) The sample (0.1–0.2 mg.), as a thin layer on glass wool, was introduced through a vacuum lock and vaporized directly into the ion source of a CEC 21-110 mass spectrometer. With this instrument, and in contrast to the earlier work,[4] all ions are focused simultaneously and recorded immediately on a photographic plate optimizing the chance of obtaining, in the first exposure, the complete spectrum (from mass 35 to 850) of the unaltered sample.

Exposure times varied from 3 to 18 min., Perfluorokerosene, admitted coincidently with the sample, provided the calibration lines required for the mass determination which is performed after taking the spectrum and not while the substance resides in the instrument,[4] a procedure much too slow to permit the measurement of the mass of many ions of a thermally decomposing substance. The accuracy of the mass measurement was within a few p.p.m. (see Table I) with the resolution set for 1:15,000. The principle of the measuring technique has been discussed previously.[9]

(9) K. Biemann, Conference on Mass Spectrometry, San Francisco, Calif., May, 1963, pp. 235–240.

(10) Such pyrolytic reactions had previously led to complications with another dimeric indole alkaloid voacamine: G. Büchi, R. E. Manning, and S. A. Monti, *J. Am. Chem. Soc.*, **85**, 1893 (1963).

TABLE I

Mass found	Error[a]	Composition	Elements lost[b]
		Vinblastine (I)	
838.4538	+2.4	$C_{48}H_{62}N_4O_9$[c]	
824.4372	+1.2	$C_{47}H_{60}N_4O_9$[d]	
811.4234	−0.3	$C_{45}{}^{13}CH_{58}N_4O_9$	
810.4219	+1.5	$C_{46}H_{58}N_4O_9$	
752.4153	+0.4	$C_{44}H_{56}N_4O_7$	$C_2H_2O_2$
751.4080	+1.0	$C_{44}H_{55}N_4O_7$	$C_2H_3O_2$
750.4014	+2.2	$C_{44}H_{54}N_4O_7$	$C_2H_4O_2$
651.3906	−0.5	$C_{40}H_{51}N_4O_4$	$C_6H_7O_5$
650.3858	+2.6	$C_{40}H_{50}N_4O_4$	$C_6H_8O_5$
591.3723	+2.6	$C_{38}H_{47}N_4O_2$	$C_8H_9O_7$
154.1219	−1.3	$C_9H_{16}NO$	$C_{37}H_{42}N_3O_8$
		VLB hydrazide (II)	
711.4198	+1.1	$C_{40}{}^{13}CH_{54}N_6O_5$	
710.4126	−2.7	$C_{41}H_{54}N_6O_5$	
651.3880	−2.8	$C_{40}H_{51}N_4O_4$	CH_3N_2O
593.3841	−1.2	$C_{38}H_{49}N_4O_2$	$C_3H_5N_2O_3$
592.3775	−4.5	$C_{38}H_{48}N_4O_2$	$C_3H_6N_2O_3$
509.2711	+3.4	$C_{32}H_{35}N_3O_3$	$C_9H_{19}N_3O_2$
154.1232	+0.5	$C_9H_{16}NO$	$C_{32}H_{36}N_5O_4$

[a] Difference in millimass units between mass found and value calculated for the elemental composition in the third column (^{12}C = 12.000000). [b] From mass 810 in case of I. [c] Dimethylation product. [d] Monomethylation product.

heating of compact sample) only mass 838 and no. 810 was observed.

Thus, the composition of VLB is $C_{46}H_{58}N_4O_9$ and the species of higher mass are most likely produced by methyl transfer from the CO_2CH_3 groups of one molecule to the nitrogen atom(s) of another followed by a Hofmann-type elimination,[10] a hypothesis strengthened by the simultaneous increase of $M-C_2H_2O_2$ corresponding to the decarbomethoxylation product of one of the methylating molecules.

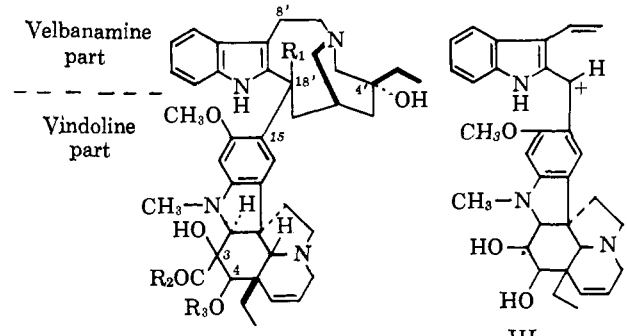
Aside from the expected elimination of CO_2CH_3 and/or $OCOCH_3$ and CH_3COOH, loss of $C_6H_8O_5$, *i.e.*, a highly oxygenated site in the molecule, is of interest. It represents the C-3, C-4 bridge analogous to the fragmentation observed with vindoline and its degradation products.[11] Without determining the elemental composition of the resulting ion, the loss of 160 mass units from VLB would also be compatible with many other processes.

Because of the thermal lability of VLB, we have determined also the masses of many peaks of the hydrazide II (which lacks the transmethylating carbomethoxy groups), some of which are listed in Table I. Particularly informative is the ion (592) which corresponds to the loss of $C_3H_6N_2O_3$, a unique combination that must contain the hydrazido group (CH_3N_2O) and two oxygen functions within two carbon atoms. This single piece of evidence requires that (i) the hydrazido group is in the highly oxygenated part, *i.e.*, at the C-3, C-4 bridge of the vindoline portion; (ii) the carbomethoxy group is thus lost from the velbanamine portion, only possible chemically if located at C-18′ or C-8′; (iii) the acetoxy group is in fact located at C-4 rather than in the velbanamine moiety as VLB loses five oxygen atoms with this bridge (see discussion above). This corroborates the independent conclusions based on chemical evidence[5] and n.m.r.[7] and demonstrates that a high resolution mass spectrum requiring much less time and material leads to the same results.

Additional information regarding the attachment of the two parts of the VLB molecule requires fragments of medium size representing the aromatic moiety of vindoline and either the indole or the piperidine part of velbanamine. Such a peak is found at m/e = 509 in the spectrum of II and is in agreement with structure III for this ion as its high oxygen content requires the presence of part of the alicyclic moiety of vindoline with the exception of the hydrazido group (only three nitrogens), and its low hydrogen content eliminates the presence of the piperidine part of velbanamine.

The fragment of mass 154 ($C_9H_{16}NO$), most abundant in velbanamine and thought to represent the piperidine portion with C-1′ and C-7′,[5] is present also in the spectra of I and II, making attachment of an aromatic ring to that region highly improbable, and agrees with a C-15, C-18′ or C-15, C-8′ bond.

The results demonstrate not only that masses in the region of mass 850 can be measured with high accuracy using *simultaneous* photographic recording[8] and that such data are particularly useful in the interpretation of



I, $R_1 = COOCH_3$; $R_2 = OCH_3$; $R_3 = COCH_3$

II, $R_1 = R_3 = H$; $R_2 = NHNH_2$

III

the spectrum of a compound containing a large number of different heteroatoms, but, more importantly, that erroneous mass spectrometric molecular weights can be obtained with substances of low volatility containing within the molecule both potential alkylating and alkylatable groups. High resolution data, carefully obtained[8] and interpreted, can eliminate this difficulty.

(12) Recipient of a fellowship from the Stiftung fur Stipendien auf dem Gebiete der Chemie (Switzerland).

DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS

P. BOMMER[12]
W. MCMURRAY
K. BIEMANN

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(11) M. Gorman, N. Neuss, and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962); K. Biemann, *Ind. Chim. Belge.*, 1319 (1962).

Vinca Alkaloids. XXI.[1] The Structures of the Oncolytic Alkaloids Vinblastine (VLB) and Vincristine (VCR)[2]

Sir:

In an earlier communication[3] partial formulas were suggested for VLB and VCR, two therapeutically use-

(1) Vinca XX: G. H. Svoboda, *Lloydia*, **26**, 243 (1963).

(2) These A.M.A. approved generic names refer to the alkaloids vincaleukoblastine and leurocristine, respectively. VLB is supplied as VELBAN® (vinblastine sulfate, Lilly) and VCR as ONCOVIN® (vincristine sulfate, Lilly).

(3) N. Neuss, M. Gorman, H. E. Boaz, and N. J. Cone, *J. Am. Chem. Soc.*, **84**, 1509 (1962).