this technique is superior to established assay methods.

The carbon-14 form of the amino acid whose activation is being studied is incubated with adenosine triphosphate, hydroxylamine and the enzyme preparation in a total volume of as little as 100 μl. Aliquots are removed at appropriate time intervals, heated briefly to destroy the enzyme and evaporated onto a line one inch from the end of a $^3/_4$ by 5 inch strip of Amberlite IR-120 ion exchange paper (sulfonic acid resin, Na^+ form, courtesy of Rohm and Haas). A sodium phosphate buffer (*p*H 7.0, 0.05 *M*) is allowed to rise by capillarity through the strip which is then dried. Under these conditions all the free unreacted neutral amino acids move with the solvent front and the hydroxamates of the neutral amino acids remain at the origin. (Other conditions permit the separation of the acidic or basic amino acids from their hydroxamates.) Comparison of the radioactivity at the two sites indicates the fraction converted and hence the rate of activation. Although the paper absorbs some of the radiation, the use of high activity L-amino acids (20 mcuries/mmole) and a thin window Geiger counter (Nuclear Chicago D-47, 45% efficiency) permits us to recognize 10^{-12} mole of hydroxamate formation in the aliquot. A typical experiment is given:

INITIAL RATES (MμMOLES/ML./HR.) C-14 HYDROXAMATE FORMATION

	+ no C-12 amino acid	+11 mM. valine	+11 mM. isoleucine	+11 mM. alloisoleucine
Valine-C-14, mM.				
0.15	96		46	44
1.0	94			
Isoleucine-C-14, mM.				
0.15	85	34		30
1.0	82			
Alloisoleucine-C-14, mM.				
0.15	1.2	0.0	0.3	
1.0	9.0			

The incubation mixtures contained in addition to the amino acids, 10 mM. adenosine triphosphate, 12 mM. Mg^{++}, 25 mM. KCl, 50 mM. tris-(hydroxymethyl)-aminomethane, 0.2 *M* sucrose and 2.0 *M* hydroxylamine. The volume was 0.23 ml., the *p*H 7.4, and the temperature 25°. Each incubation flask contained 0.05 ml. of a dilute extract of alumina ground *E. coli*. Aliquots of 25 μl. were removed for analysis at zero, 30, 60 and 120 minutes. The rate of hydroxamate formation was linear except when the substrate was approaching exhaustion.

Similar assays can be devised for any system in which the starting material and product can be caused to differ markedly in charge; *e.g.*, the conversion of glucose into glucose phosphate, of acetate into acetohydroxamate, or the pyrophosphate exchange into adenosine triphosphate.

HUNTINGTON MEMORIAL LABORATORIES
OF HARVARD UNIVERSITY ROBERT BERNER LOFTFIELD
MASSACHUSETTS GENERAL HOSPITAL
BOSTON, MASS.
MARINE BIOLOGICAL LABORATORY
WOODS HOLE, MASS. ELIZABETH ANN EIGNER

RECEIVED JULY 6, 1959

VINCA ALKALOIDS. III.[1] CHARACTERIZATION OF LEUROSINE AND VINCALEUKOBLASTINE, NEW ALKALOIDS FROM *VINCA ROSEA LINN.*

Sir:

Leurosine,[2] a new alkaloid from *Vinca rosea Linn.*, was described recently, but no empirical formula was assigned.[2] Independently Noble, Beer and Cutts have reported the physical and biological properties of another new alkaloid, vincaleukoblastine.[3,4]

In view of the unusual properties of these two alkaloids,[2,3,4] we wish to present the analytical and physical data which led to the establishment of empirical formulas for vincaleukoblastine and leurosine and indicate their close structural relationship.

Vincaleukoblastine sulfate[5] melted at 284–285°, $[\alpha]^{26}D$ −28° (CH_3OH). Calcd. for $C_{46}H_{58}O_9N_4 \cdot H_2SO_4 \cdot H_2O$: C, 59.59; H, 6.74; O, 24.16; N, 6.04; S, 3.46. Found: C, 59.68; H, 6.72; O, 24.27; N, 6.19; S, 3.37. The free base, recrystallized from ether, formed a stable etherate, loss of solvent at 180–182°, m.p. 201–211°, $[\alpha]^{26}D$ +42° ($CHCl_3$). Calcd. for $C_{46}H_{58}O_9N_4 \cdot (C_2H_5)_2O$: C, 67.85; H, 7.74; O, 18.09; N, 6.32; mol. wt., 885. Found: C, 67.89, 67.93; H, 7.63, 7.76; O, 18.08; N, 6.38, 6.43; mol. wt., 887.8 (X-ray data). Ether of solvation was demonstrated by vapor phase chromatography and a band at 8.4 μ in the infrared disappearing on evaporation of a chloroform solution of the etherate. The base from methanol melted at 211–216°, calcd. for $C_{46}H_{58}O_9N_4 \cdot 2CH_3OH \cdot H_2O$: C, 64.55; H, 7.68; N, 6.27; mol. wt., 894. Found: C, 64.11; H, 7.49; N, 6.36; mol. wt., 889 ± 5 (electrometric titration, H_2O; pK'_a 5.4, 7.4). After drying at 180° (1 min.), calcd. for $C_{46}H_{58}O_9N_4$: C, 68.12; H, 7.21; O, 17.75; N, 6.90; weight loss, 9.19. Found: C, 68.15; H, 7.44; O, 18.05; N, 6.65; weight loss, 8.81. Vincaleukoblastine formed a dihydrochloride dihydrate, m.p. 244–246° (dec.). Calcd. for $C_{46}H_{58}O_9N_4 \cdot 2HCl \cdot 2H_2O$: C, 60.06; H, 7.01; O, 19.13; N, 6.09; Cl, 7.71. Found: C, 60.36, 59.95; H, 7.24, 7.18; O, 19.04; N, 5.94; Cl, 7.37.

Leurosine[2] was recrystallized from acetonitrile, m.p. 202–205° (dec.) (loss of solvent at 172–175°), $[\alpha]^{26}D$ +72° ($CHCl_3$). Calcd. for $C_{46}H_{58}O_9N_4 \cdot 8H_2O$: mol. wt., 955.09. Found: mol. wt., 955.3 ± 1% (X-ray data); 932 ± 10 (electrometric titration, pK'_a 5.5 and 7.5 in water). After drying at 130° *in vacuo*, weight loss calcd.: 15.09. Found: 15.60. Calcd. for $C_{46}H_{58}O_9N_4$: C, 68.12; H, 7.21; O, 17.75; N, 6.90. Found: C, 68.11, 67.88; H, 7.30, 7.45; O, 17.34, 18.05; N, 7.10, 6.93. The sulfate from ethanol, $[\alpha]^{26}D$ −8.3 (CH_3OH), m.p. 238–242° (dec.), was dried at 130° *in vacuo*. Calcd. for $C_{46}H_{58}O_9N_4 \cdot H_2SO_4$: C, 60.77;

(1) Vinca Alkaloids II, M. Gorman et al., *J. Am. Pharm. Assoc. Sci. Ed.*, **48**, 256 (1959).

(2) G. H. Svoboda, *J. Am. Pharm. Assoc. Sci. Ed.*, **47**, 834 (1959).

(3) R. L. Noble, C. T. Beer and J. H. Cutts, *Ann. N. Y. Acad. Sci.*, **76**, 882 (1958).

(4) R. L. Noble, C. T. Beer and J. H. Cutts, *Biochemical Pharmacology*, **1**, 347 (1958).

(5) The alkaloid sulfate was first tentatively formulated as a $C_{46}H_{58}N_2O_7 \cdot 1/2H_2SO_4$ compound.[3] The scarcity of the material did not allow at that time the corroboration of this formula. The alkaloid and its derivatives solvate readily and retain tenaciously solvents of crystallization.

H, 6.65; O, 22.87; N, 6.16; S, 3.52. Found: C, 60.90, 61.23; H, 6.52, 6.52; O, 22.86; N, 6.07, 6.15; S, 3.43. Analytical data for leurosine and vincaleukoblastine are in agreement with a tentative formulation as isomeric $C_{46}H_{58}O_9N_4$ compounds.[6] Their ultraviolet spectra are superimposable: λ_{max}^{EtOH} 214 mμ (log a_M 4.74), 259 mμ (log a_M 4.22), and λ_{min}^{EtOH} 246 mμ (log a_M 4.14); shoulders at 288 mμ (log a_M 4.15) and 296 mμ (log a_M 4.12).

The close structural relationship of these two alkaloids is demonstrated further by their essentially identical infrared spectra. The major differences occur in the hydroxyl region of vincaleukoblastine with additional bands at 2.80 and 9.91 μ.[7]

(6) An alternate C_{18} formulation was discarded on the basis of electrometric titrations, carbon–oxygen ratios and functional group analyses as presented in the following communication.

(7) For these spectra, see communication IV, p. 4745.

(8) Medical Research Associate of the National Research Council of Canada.

LILLY RESEARCH LABORATORIES
INDIANAPOLIS 6, INDIANA
N. NEUSS
M. GORMAN
G. H. SVOBODA
G. MACIAK

DEPARTMENT OF MEDICAL RESEARCH
UNIVERSITY OF WESTERN ONTARIO
LONDON, CANADA
C. T. BEER[8]

RECEIVED JULY 6, 1959

THE DIRECT FLUORINATION OF UREA: THE SYNTHESIS AND PROPERTIES OF DIFLUORAMINE

Sir:

The direct fluorination of urea at 0° yields a complex yellow, corrosive liquid which contains up to 16% active fluorine (to HI) and about 45–55% total fluorine. On solution in water, ammonium fluoride, biurea and unidentified refractory solids are obtained.[1] Distillation of the liquid from Kel-F or polyethylene into glass yields, in the more volatile fraction, CO_2, SiF_4, HNCO, COF_2 and difluoramine, HNF_2.[2] As high as 15% of the original fluorine has been recovered as difluoramine.

Ruff and Staub[3] first reported the preparation of difluoramine but gave no analysis and erroneous physical properties. They also reported that it did not react with aqueous hydriodic acid, which we observed. Therefore, we agree with Kennedy and Colburn[2] that the material described by Ruff and Staub was not difluoramine. Our vapor pressure data agree with Kennedy and Colburn's within experimental error and the infrared spectra are identical. However, our mass spectrum and melting point do differ.

We found that gaseous difluoramine loses hydrogen on contact with various solids to form the recently reported tetrafluorohydrazine.[4] With lithium hydride as a catalyst, yields of 70% are obtained easily. When chilled to −196°, solid difluoramine tends to detonate spontaneously. Chilling only to −142° and working with small samples, minimizes this tendency, but the violence of the reaction requires adequate precautions be taken. Physical properties we determined are listed in Table I.

(1) O. Glemser and H. Ludemann, *Z. anorg. allgem. Chem.*, **286**, 168 (1956).

(2) A. Kennedy and C. Colburn, THIS JOURNAL, **81**, 2906 (1959).

(3) O. Ruff and L. Staub, *Z. anorg. allgem. Chem.*, **198**, 32 (1931).

(4) C. B. Colburn and A. Kennedy, THIS JOURNAL, **80**, 5004 (1958).

TABLE I

THE PHYSICAL PROPERTIES OF DIFLUORAMINE

Melting point, °C.	−116 ± 3°
Boiling point, °C.	−23.6°
Density	$d = 1.424 - 0.00202t$
Trouton constant	23.7

Difluoramine was identified by its molecular weight (calculated for HNF_2, 53.02; observed, 54) and its almost instantaneous and quantitative reaction with 0.75 *N* HI according to the equation

$$HNF_2 + 4HI \longrightarrow 2I_2 + NH_4F + HF$$

The mass spectrum taken with a CEC Model 103C mass spectrometer (Table II) is consistent with the above formulation. All of these peaks are reproducible on different samples.

TABLE II

MASS SPECTRUM OF DIFLUORAMINE

m/e	Pattern coef.	+ Ion
14	19.37	N
15	10.50	HN
19	6.89	F
20	1.95	HF
28	1.61	N_2
33	34.35	NF
34	100.00	HNF
52	1.5	NF_2
53	66.97	HNF_2

Sensitivity *n*-butane *m/e* 43 = 69.89 div./μ.
Sensitivity HNF_2 *m/e* 34 = 23.12 div./μ.
Ionizing voltage 70 v.
Ionizing current 10 μa.

The authors are indebted to the Office of Naval Research for support of this work. The mass spectral determination was performed by Mr. Mario Stevens of this laboratory. Mr. Martin Epstein participated in the initial work.

CHEMICAL RESEARCH GROUP
ROCKETDYNE
CANOGA PARK, CALIFORNIA
EMIL A. LAWTON
JOHN Q. WEBER

RECEIVED MAY 11, 1959

UNSATURATED MACROCYCLIC COMPOUNDS. XI.[1] CYCLOTETRACOSA-1,3,7,9,13,15,19,21-OCTAENE-5,11,-17,23-TETRAYNE AND CYCLOTETRACOSA-1,3,5,-7,9,11,13,15,17,19,21,23-DODECAENE

Sir:

We wish to report the synthesis of the completely conjugated 24-membered ring cyclic systems named in the title.

Cyclotetracosa-1,3,7,9,13,15,19,21-octayne (I) (the cyclic "tetramer" of 1,5-hexadiyne)[2] on treatment with potassium *t*-butoxide in *t*-butanol–benzene at 90° for 30 minutes underwent a similar rearrangement to that of the corresponding "trimer."[3] The product, formed in *ca.* 40% yield, was obtained as dark purple prisms from ether (red in solution), which decomposed when heated.

(1) Part X, F. Sondheimer and Y. Gaoni, THIS JOURNAL, in press.

(2) F. Sondheimer, Y. Amiel and R. Wolovsky, *ibid.*, **79**, 4247 (1957).

(3) F. Sondheimer and R. Wolovsky, *ibid.*, **81**, 1771 (1959).