over 150 g. of neutral alumina. After initial elution with benzene and benzene–3% ethyl acetate, the use of benzene–6% ethyl acetate removed the bulk of the reaction products. From these latter cuts was obtained a cream-colored crystalline mass which was readily recrystallizable from methanol to give 177 mg. of crude adduct, m.p. 113–120°, representing a total yield of 60% based on starting benzocalicene ester.

Four recrystallizations of this crude adduct from ether–petroleum ether gave 66 mg. of *trimethyl 1,2-dipropylphenanthrene-3,4,9-tricarboxylate*, m.p. 122–123°, exhibiting principal infrared maxima (KBr) at 5.74, 5.80, 6.96, 7.83, 7.96, and 8.33 μ. The triester was dried at 70° and oil pump vacuum for 30 min. prior to analysis.

Anal. Calcd. for $C_{26}H_{28}O_6$: C, 71.54; H, 6.47. Found: C, 72.00; H, 6.66.

Crystallization of the combined mother liquors gave, first, another 15 mg. of the slightly impure triester described above; the subsequent crop gave 82 mg. of the mixture of isomeric esters. This crop had m.p. 113–126°, and an ultraviolet spectrum substantially identical with that of the pure triester, m.p. 122–123°. A combustion analysis of this mixture was slightly low in carbon.

Anal. Calcd. for $C_{26}H_{28}O_6$: C, 71.54; H, 6.47. Found: C, 70.69; H, 6.46.

Hydride Reduction of Trimethyl 1,2-Dipropylphenanthrene-3,4,9-tricarboxylate. A solution of the 123° triester (6 mg.) in freshly distilled, peroxide-free tetrahydrofuran (3.0 ml.) was stirred under nitrogen with 150 mg. of lithium aluminum hydride. The temperature of the suspension was warmed to 55–60° and the reduction was monitored by ultraviolet spectrophotometry. After 1 hr. at this temperature, the spectrum remained constant. Semiquantitative assay of an aliquot in acidified methanol gave the following maxima: 363 mμ (log ϵ 2.4), 347 (2.4), 313 (2.8), 302 (2.9), and 263 (4.6). Because of scarcity of sample this product was not further investigated.

Acknowledgment. It is a pleasure to acknowledge the assistance of W. Fulmor and G. Morton (Lederle) and of J. Lancaster and R. Murray of the Stamford Laboratories, American Cyanamid Company, for the spectra and dipole moments reported in this paper. We are indebted to C. Pidacks and his staff for the chromatographic separations performed in this investigation, to L. Binovi for the preparation of intermediates, and to L. Brancone and his group for the analytical determinations. Finally, we are grateful to Dr. J. J. Hlavka for first calling to our attention the possibility of phenanthrene formation in the cycloaddition of dimethyl acetylenedicarboxylate to the benzocalicene system.

Biosynthesis of the *Vinca* Alkaloids. I. Feeding Experiments with Tryptophan-2-C^{14} and Acetate-1-C^{14} [1]

Edward Leete,[2] Afzal Ahmad,[3] and Ivan Kompis[4]

Contribution from the School of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455 *Received March 13, 1965*

Radioactive vindoline was isolated from Vinca rosea plants which had been fed DL-*tryptophan-2-*C^{14}. *A systematic degradation of this alkaloid established that essentially all the activity was located at C-10, substantiating the hypothesis that tryptophan is a most probable precursor of this type of dihydroindole alkaloid. Administration of sodium acetate-1-*C^{14} *to the same species resulted in the formation of radioactive vindoline, catharanthine, and ajmalicine. Partial degradations were carried out on these alkaloids and the results obtained indicate that acetate is not serving as a direct precursor for the nontryptophan-derived portion of these alkaloids.*

The plant *Vinca rosea* Linn.[5] has been extensively investigated in the last few years because it has been found to contain potent oncolytic agents. The major alkaloid found in the leaves is vindoline (I). The pentacyclic ring system found in this compound[6] has been previously encountered in the alkaloid aspidospermine,[7] and a large group of alkaloids is now known with this type of skeleton.[8]

Since the β-(2-aminoethyl)indole moiety is present in vindoline, it has generally been accepted that this part of the alkaloid is derived from tryptophan or its decarboxylation product tryptamine.[9] We have now concluded that tryptophan is indeed a most probable direct precursor of this part of the vindoline molecule

(1) Some of this work was presented at the Third International Symposium on the Chemistry of Natural Products, Kyoto, Japan, April 1964. This investigation was supported by Research Grant CA-5336 from the U. S. Public Health Service.

(2) Alfred P. Sloan Foundation Fellow.

(3) On leave of absence from the Pakistan Atomic Energy Commission.

(4) On leave of absence from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia.

(5) *Cf.* G. H. Svoboda, I. S. Johnson, M. Gorman, and N. Neuss, *J. Pharm. Sci.*, **51**, 707 (1962), for a review on the numerous alkaloids found in this plant, the correct botanical name of which is apparently *Catharanthus roseus* G. Don.

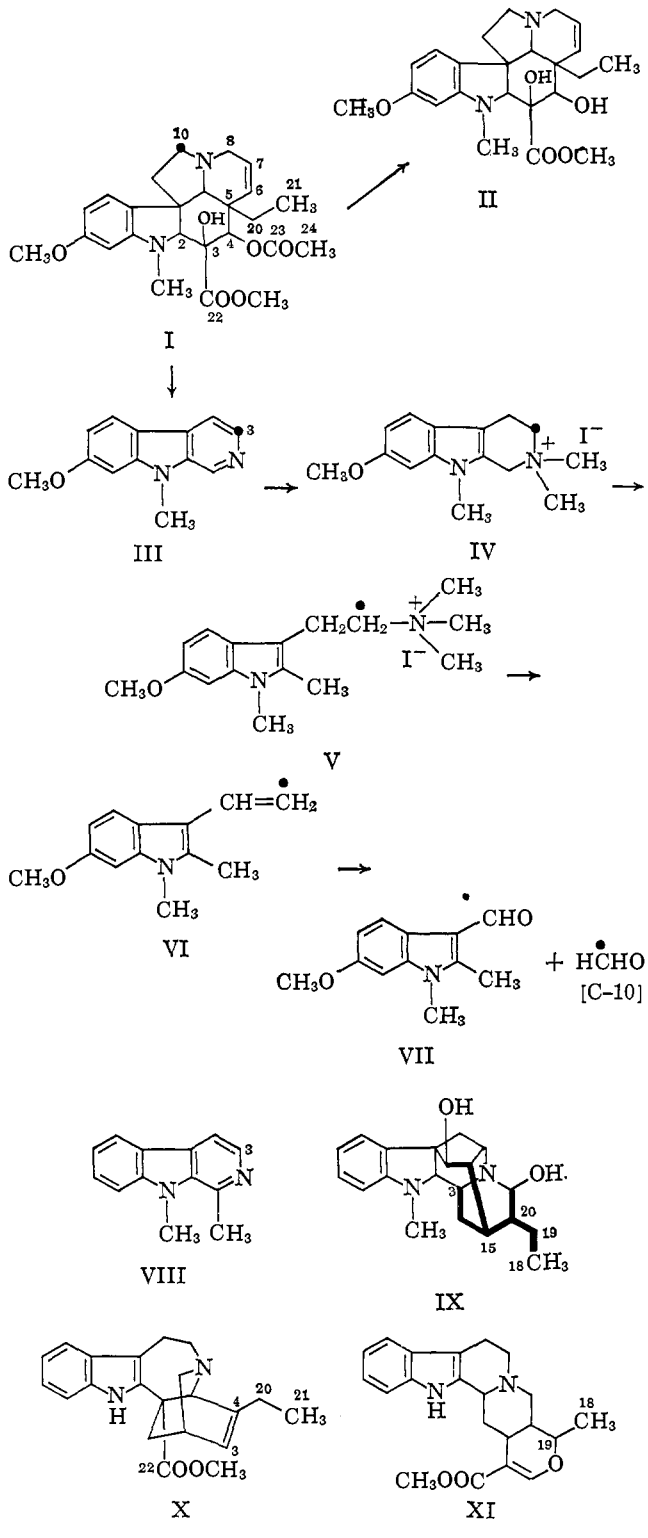
(6) (a) M. Gorman, N. Neuss, G. H. Svoboda, A. J. Barnes, and N. J. Cone, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 256 (1959); (b) M. Gorman, N. Neuss, and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962).

(7) J. F. D. Mills and S. C. Nyburg, *Tetrahedron Letters*, **No. 11**, 1 (1959); H. Conroy, P. R. Brook, and Y. Amiel, *ibid.*, **No. 11**, 4 (1959).

(8) *Cf.* "The Alkaloids, Chemistry and Physiology," Vol. VIII, R. H. F. Manske, Ed., Academic Press Inc., New York, N. Y., 1965.

(9) It has been suggested by E. Wenkert, *J. Am. Chem. Soc.*, **84**, 98 (1962), that the aspidospermine type alkaloids are formed not from tryptophan, but from its progenitors, namely derivatives of anthranilic acid.

Scheme I



by tracer experiments. DL-Tryptophan-2-C^{14} was fed to 4-month-old *Vinca rosea* plants by means of cotton wicks inserted into the stems. Two weeks after administration of the tracer the plants were harvested and radioactive vindoline (0.32% incorporation) was isolated by established procedures.[10] Other workers[11,12] have also obtained radioactive vindoline from tryptophan-2- or -3-C^{14}; however, no degradations were carried out to determine the location of activity. We have now degraded the radioactive vindoline derived from tryptophan-2-C^{14} by the scheme illustrated in Scheme I. The expected position of labeling was at C-10 and the initial key step in the degradation was the formation of *ind*-N-methylnorharmine (III, 7-methoxy-9-methyl-β-carboline) by a soda lime distillation of vindoline.[6b] The yield in this reaction is poor; however, the original vindoline had a sufficiently high specific activity that considerable dilution with inactive material could be carried out at this stage in the degradation. It seems reasonable to assume that this β-carboline is formed by a migration of C-19 to C-2, and that C-10 of vindoline becomes C-3 of the β-carboline derivative. Subsequent steps in the degradation were routine since a systematic degradation had been previously carried out on the related compound, 1,9-dimethyl-β-carboline-3-C^{14}.[13] The β-carboline III was converted to its methiodide which was reduced with sodium borohydride to 7-methoxy-2,9-dimethyl-1,2,-3,4-tetrahydro-β-carboline. Emde reduction on the 2-methiodide of this compound afforded 6-methoxy-1,2-dimethyl-N,N-dimethyltryptamine. The methiodide (V) of this tryptamine derivative was also prepared by an unambiguous route from 6-methoxy-2-methylindole[14] using the tryptamine synthesis of Speeter and Anthony.[15] Hofmann degradation on the methiodide V yielded 6-methoxy-1,2-dimethyl-3-vinylindole (VI) which was oxidized with osmium tetroxide and sodium metaperiodate to formaldehyde (collected as its dimedone derivative) and 6-methoxy-1,2-dimethylindole-3-carboxaldehyde (VII). The activities of the degradation products are recorded in Table II. The aldehyde VII was inactive, but the formaldehyde–dimedone derivative and all the other intermediates in this degradative scheme had activities almost equal to that of vindoline, indicating that most of the activity was located at C-10.

We then turned our attention to the nontryptophan-derived portion of vindoline. The origin of the C-10 unit, comprising carbon atoms 3–8 and 19–22, has been the subject of considerable discussion.[9,16] It is relevant at this point to discuss our work on the biosynthesis of ajmaline (IX). In 1962 we reported[17] that the ajmaline isolated from *Rauwolfia serpentina* plants which had been fed mevalonic acid-2-C^{14} or tyrosine-2-C^{14} was completely inactive. We considered that these results rendered unlikely both the "Woodward fission hypothesis"[18] and the "monoterpene hypothesis" of Thomas[16] and Wenkert[9] for the origin of the branched C-9 unit (indicated with heavy lines in formula IX). We also fed alanine-2-C^{14} to test Wenkert's "prephenic acid hypothesis."[19] However, this experiment may not be valid since we had assumed that pyruvate formed from alanine by

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

(11) Unpublished work of R. McMahon and M. Gorman of the Lilly Research Laboratories, Indianapolis, cited in ref. 6b, footnote 10, and also in a personal letter of R. McMahon to E. Leete (May 4, 1961).

(12) D. Gröger, K. Stolle, and K. Mothes, *Tetrahedron Letters*, 2579 (1964).

(13) E. Leete, *J. Am. Chem. Soc.*, 82, 6338 (1960).

(14) E. Spath and O. Brunner, *Ber.*, 58, 518 (1925).

(15) M. E. Speeter and W. C. Anthony, *J. Am. Chem. Soc.*, 76, 6208 (1954).

(16) R. Thomas, *Tetrahedron Letters*, 544 (1961).

(17) E. Leete, S. Ghosal, and P. N. Edwards, *J. Am. Chem. Soc.*, 84, 1068 (1962).

(18) R. B. Woodward, *Angew. Chem.*, 68, 13 (1956).

(19) E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, 81, 1474 (1959); E. Wenkert, *Experientia*, 15, 165 (1959).

transamination would be incorporated into the side chain of prephenic acid; the actual precursor of the side chain of prephenic acid is phosphoenolpyruvate which is not readily formed from pyruvic acid *in vivo*.[20] These negative results led us to feed sodium acetate-1-C^{14} to *R. serpentina*. Ajmaline having a satisfactory activity was obtained from the plant, and partial degradation indicated that approximately half of the total activity of the alkaloid was located at C-3 and C-19 and equally distributed between these positions. On the basis of these results we suggested that the branched C-9 unit in ajmaline was formed by the linear combination of three acetate units followed by reaction at C-15 with malonic acid and at C-20 with a one carbon unit. Later work[21] apparently substantiated this hypothesis. However, Battersby, also studying the biosynthesis of ajmaline, reported results[22] which were not consistent with ours. Degradations carried out on radioactive ajmaline obtained from *R. serpentina* plants which had been fed acetate-1-C^{14} indicated that activity in the alkaloid was more or less uniformly distributed. We must now also report that we have obtained radioactive ajmaline, which is apparently uniformly labeled, from *R. serpentina* plants which had been fed sodium acetate-1-C^{14}.[23] These recent results have caused the senior author to be concerned about the authenticity of our earlier published work[17,21]; however, no obvious deception has been detected and the matter is still under investigation.

The nontryptophan-derived portion of vindoline can also be hypothetically constructed from three acetate units, malonic acid, and a one-carbon unit. In addition vindoline contains an O-acetyl group. We therefore fed sodium acetate-1-C^{14} to *V. rosea* plants and after 2 weeks radioactive vindoline, catharanthine (X) and ajmalicine (XI) were isolated.[24] The last two alkaloids also contain C-10 units whose structures can be readily accommodated by the "acetate hypothesis." Partial degradations have been carried out on these alkaloids and the results are recorded in Table II. Acid hydrolysis of vindoline afforded desacetylvindoline (II) and acetic acid. This acetic acid, assayed as 1-acetamidonaphthalene,[25] contained 39% of the original activity of the vindoline. A Schmidt reaction on the acetic acid yielded carbon dioxide assayed as barium carbonate and methylamine collected as N-methylbenzamide. About 90% of the activity of the acetic acid was located in the carboxyl group indicating that the O-acetyl group is produced, as expected, from acetic acid with little randomization of activity. A Kuhn–Roth oxidation of desacetylvindoline afforded a mixture of acetic and propionic acid, derived from the C-ethyl group and the adjacent carbon at position 5. These acids were further degraded by Schmidt reactions, and essentially uniform labeling was found at C-5, -20, and -21 (4% at each). A Kuhn–Roth oxidation of catharanthine yielded acetic and propionic acids derived from C-4, -20, and -21, and there was uniform labeling at these three carbons (5–6% at each). Catharanthine was hydrogenated to 3,4-dihydrocatharanthine which was hydrolyzed with methanolic potassium hydroxide. Acidification resulted in decarboxylation and the formation of epiibogamine.[26] The evolved carbon dioxide was collected as barium carbonate having 7.5% of the total activity of the catharanthine. The amount of ajmalicine obtained from the plant was small, but a Kuhn–Roth oxidation yielded acetic acid which was also uniformly labeled. Radioactive vindoline and catharanthine were also isolated from plants which had been allowed to grow in contact with acetate-1-C^{14} for only 48 hr. Most of the activity (81%) of the vindoline was present in the O-acetyl group, in which there was practically no randomization of activity. Lack of material and low activities prevented further degradation of the desacetylvindoline. Degradation of the catharanthine indicated that there was uniform labeling in C-4, -20, and -21.

Our present results thus apparently eliminate the "acetate hypothesis" for the origin of the C-10 unit in vindoline, catharanthine, and ajmalicine. The "monoterpene hypothesis" also seems unlikely since mevalonic acid, the precursor of terpenes, is derived from acetate, and specific labeling would be expected in the derived terpene.[27]

The low level of activity found in the C-ethyl groups of vindoline and catharanthine, and in the C-methyl group of ajmalicine, seems to indicate that all carbon atoms, and not just those present in the C-10 units, have become labeled after feeding acetate-1-C^{14}. The precursors of tryptophan are serine, ribose, and anthranilic acid which is formed from shikimic acid.[28] This means that the label of acetate-1-C^{14} has entered the general metabolic pool involving carbohydrates, and it is probable that the precursors of the C-10 units are closely related to intermediates involved in carbohydrate metabolism. We are actively examining such intermediates as precursors of the elusive, umbrageous C-10 unit.

Experimental[29]

Administration of Tracers to Vinca rosea Plants and Isolation of the Alkaloids. Details of the amounts of tracer fed and the yields of alkaloids are recorded in Table I. The alkaloids were isolated according to the procedure of the Eli Lilly group,[10] modified for work on a smaller scale. The following is a typical work-up.

(20) C. Gilvarg and K. Bloch, *J. Biol. Chem.*, **193**, 339 (1951); D. B. Sprinson, *Advan. Carbohydrate Chem.*, **15**, 235 (1960); see, however, I. Krimsky, *J. Biol. Chem.*, **234**, 232 (1959).

(21) E. Leete and S. Ghosal, *Tetrahedron Letters*, 1179 (1962).

(22) A. R. Battersby, R. Binks, W. Laurie, G. V. Parry, and B. R. Webster, *Proc. Chem. Soc.*, 369 (1963).

(23) Unpublished work of J. R. Gear and E. Leete. Hydrolysis of radioactive reserpine isolated from the same plant yielded reserpic acid and trimethoxybenzoic acid having relative specific activities of 63.5 and 31.5%, respectively, indicative of uniform labeling.

(24) Gröger, *et al.*,[12] fed acetate-1- and -2-C^{14} to *V. rosea* and isolated radioactive vindoline, vindolinine, and catharanthine.

(25) *Cf.* E. Leete, H. Gregory, and E. G. Gros, *J. Am. Chem. Soc.*, 87, 3475 (1965), for the preparation of this derivative on a small scale. This compound and the corresponding one from propionic acid are most convenient for accurate liquid scintillation counting in toluene solution.

(26) M. Gorman, N. Neuss, and N. J. Cone, *ibid.*, 87, 93 (1965).

(27) A. R. Battersby and G. V. Parry, *Tetrahedron Letters*, 787 (1964), found that the β-sitosterol isolated from *R. serpentina* plants which had been fed acetate-1-C^{14} was labeled at specific positions while the ajmaline isolated from the same plants was uniformly labeled.

(28) L. M. Henderson, R. K. Gholson, and C. E. Dalgliesh, in "Comparative Biochemistry," Vol. IV, M. Florkin and M. S. Mason, Ed., Academic Press Inc., New York, N. Y., 1962, p. 245.

(29) Melting points are corrected. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation spectrometer, Model 724, using as solvents either toluene or dioxane–water with the usual scintillators (*cf.* A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141 (1963)). Microanalyses were carried out by Mrs. Olga Hamerston, Mr. T. S. Prokopov, and their assistants, at the University of Minnesota.

Table I. Feeding Experiments with *Vinca rosea* Plants

Experiment No.	1	2	3
Precursor fed	DL-Trypto-phan-2-C^{14}[a]	Sodium acetate-1-C^{14}[b]	
Wt., mg.	14	8.5	5.0
Activity, mcurie	0.2	1.0	1.0
Time of feeding	2 weeks	2 weeks	2 days
Fresh wt. of plants, g.	496	452	530
Date of feeding	Aug. 1963	Aug. 1963	Aug. 1964
Vindoline			
Wt., mg.	46.8	54.6	62.3
Activity, d.p.m./mmole	1.37×10^7	2.60×10^6	1.11×10^6
Incorporation, %	0.32	0.014	0.007
Catharanthine			
Wt., mg.	Not isolated	41.6	24.4
Activity, d.p.m./mmole		1.61×10^6	2.68×10^5
Incorporation, %		0.0091	0.0009
Ajmalicine			
Wt., mg.	Not isolated	3.34	Not isolated
Activity, d.p.m./mmole		6.93×10^5	
Incorporation, %		0.0003	

[a] Purchased from Tracerlab, Inc., Waltham, Mass., and fed as a dilute solution in acetic acid. [b] Purchased from New England Nuclear Corp., Boston, Mass.

The fresh plants from experiment 2 (wet weight 452 g.) were ground in a 1-gal. Waring blendor with a mixture of chloroform (2 l.) and concentrated ammonia (100 ml.). After standing for several days the mixture was filtered through cloth and the two layers were separated. The aqueous solution having a total activity of 8.71 $\times 10^7$ d.p.m. was extracted with an additional amount of chloroform. The combined chloroform extracts were evaporated to dryness *in vacuo* and the residue was dissolved in benzene (100 ml.) which was then extracted with 1% hydrochloric acid (six 50-ml. portions). The acid extract was made basic with ammonia and extracted with benzene (six 100-ml. portions). Evaporation of the dried (sodium sulfate) benzene extract yielded the crude alkaloids (0.71 g., 6.3×10^6 d.p.m.). The alkaloids were dissolved in 2% tartaric acid (20 ml.) and extracted with benzene (twelve 25-ml. portions). The benzene extract was concentrated to 50 ml. and extracted with 2% tartaric acid (four 25-ml. portions). This tartaric acid extract was brought to a pH of 3 with sodium hydroxide and then extracted with 1,2-dichloroethane (five 25-ml. portions). Evaporation of this organic extract yielded a residue (164.3 mg.) which was dissolved in benzene and chromatographed on alumina (Alcoa, Grade F-20, deactivated by shaking 100 g. with 3 ml. of 10% acetic acid). The column was eluted successively with benzene, benzene–chloroform (3:1), and finally pure chloroform. The fractions were monitored by thin layer chromatography.[30] Fractions containing vindoline were combined and crystallized from ether, affording vindoline (54.6 mg.), m.p. 164–165°. For degradation this material was diluted with inactive vindoline and crystallized to constant activity from ethyl acetate–ether.

The aqueous solution having a pH of 3, from which the vindoline had been extracted, was made basic with ammonia and extracted with dichloroethane. Evaporation of this extract yielded a residue (30.3 mg.) which was subjected to chromatography on an inclined glass plate containing a relatively thick layer of dry neutral Woelm alumina (activity III). Development of the plate was carried out with benzene–chloroform (3:1), and ajmalicine was found in a zone having an R_f of 0.3–0.4. Extraction of this zone with methanol afforded ajmalicine (3.34 mg.), the amount being estimated by its absorption in the ultraviolet. Inactive ajmalicine was added and ajmalicine crystallized to constant activity from methanol–water.

The original aqueous 2% tartaric acid from which the vindoline and ajmalicine fractions had been removed with benzene was extracted with dichloroethane

(30) N. J. Cone, R. Miller, and N. Neuss, *J. Pharm. Sci.*, **52**, 688 (1963). We thank M. Gorman of Eli Lilly and Co. for generous supplies of vindoline and other *Vinca* alkaloids.

Table II. Degradation Products of the Alkaloids Isolated from *Vinca rosea*

	Sp. act., d.p.m./mmole $\times 10^{-5}$	Rel. act.		
(a) Vindoline Derived from DL-Tryptophan-2-C^{14}				
Vindoline (I)	137	100		
ind-N-Methylnorharmine (III)	131	95		
6-Methoxy-1,2-dimethyl-N,N-dimethyltryptamine methiodide (V)	131	95		
6-Methoxy-1,2-dimethylindole-3-carboxaldehyde (VII)	<2	<2		
Formaldehydedimedone [C-10]	120	88		
	Feedings: 2-week	2-day	2-week	2-day
(b) Vindoline Derived from Sodium Acetate-1-C^{14}				
Vindoline (I)	26.0	11.1	100	100
O-Acetylvindoline[6a]	26.1	...	100	...
Desacetylvindoline (II)	16.5	1.9	63	17
1-Acetamidonaphthalene[a]	10.2	9.05	39	81
Barium carbonate[b] [C-23]	9.0	8.8	34.4	79
N-Methylbenzamide[b] [C-24]	0.55	0.15	2.1	1.4
1-Acetamidonaphthalene[c]	1.9	...	7.5	...
Barium carbonate[b] [C-20]	1.2	...	4.6	...
1-Propionamidonaphthalene[c]	2.9	...	11.1	...
Barium carbonate[d] [C-5]	1.0	...	3.8	...
(c) Catharanthine Derived from Sodium Acetate-1-C^{14}				
Catharanthine hydrochloride	16.1	2.68	100	100
Catharanthine hemisulfate[6a]	16.2	...	100	...
Epiibogamine	14.2	...	88	...
Barium carbonate[e] [C-22]	1.2	...	7.5	...
1-Propionamidonaphthalene[f]	2.7	0.36	17	13.4
Barium carbonate[d] [C-4]	0.90	0.12	5.6	4.5
N-Ethylbenzamide[d]	...	0.21	...	7.8
1-Acetamidonaphthalene[f]	1.9	...	12	...
Barium carbonate[b] [C-20]	0.9	0.13	5.6	4.8
N-Methylbenzamide[b] [C-21]	0.97	0.11	6.0	4.1
(d) Ajmalicine Derived from Sodium Acetate-1-C^{14}				
Ajmalicine (XI)	6.93	...	100	...
1-Acetamidonaphthalene[g]	0.82	...	12	...
Barium carbonate[b] [C-19]	0.47	...	6.8	...

[a] Derivative of the acetic acid obtained from the O-acetyl group. [b] Obtained by a Schmidt reaction on the previously mentioned acetic acid. [c] Derivatives of the acetic and propionic acids obtained by the Kuhn–Roth oxidation of desacetylvindoline. [d] Obtained by a Schmidt reaction on the previously mentioned propionic acid. [e] Obtained from the carboxyl group of catharanthine. [f] Derivatives of the acetic and propionic acids obtained by the Kuhn–Roth oxidation of catharanthine. [g] Derivative of the acetic acid obtained by the Kuhn–Roth oxidation of ajmalicine.

(five 25-ml. portions). The residue (307 mg.) obtained on evaporation was dissolved in benzene and chromatographed on Woelm alumina (activity III). Catharanthine (41.6 mg.) was present in fractions eluted with benzene–chloroform (3:1). The combined fractions were evaporated and the residue was dissolved in 0.1 ml. of concentrated hydrochloric acid. Water was removed by adding benzene and again evaporating to dryness. The residue was crystallized from methanol–ether, affording colorless prisms of catharanthine hydrochloride, m.p. 185–190° dec.

Soda Lime Fusion of Vindoline. Vindoline (495 mg.) was intimately mixed in a mortar with soda lime (10 g.) and dried *in vacuo* at 100° for 1 day. The mixture was placed in a conical flask, covered with glass wool, and heated at 325° for 10 hr. in a slow nitrogen stream. Some oil distilled onto the glass wool. After cooling the contents of the flask was extracted with benzene. The filtered benzene solution was concentrated to 50 ml. and extracted with 0.5% hydrochloric acid (four 25-ml. portions). The acidic solution was made alkaline with ammonia and extracted with benzene. Evaporation of this benzene extract yielded a residue (45.6 mg.) which was subjected to preparative thin layer chromatography on alumina. Development with benzene containing 1% methanol yielded a zone strongly fluorescent in ultraviolet light at the same R_f (0.2–0.3) as authentic *ind*-N-methylnorharmine. This zone was extracted with chloroform and the residue obtained on evaporation of the solvent sublimed *in vacuo* (100° at 0.005 mm.). The yield of *ind*-N-methylnorharmine (1.48 mg.) was estimated from its ultraviolet absorption in 95% ethanol: λ_{max} 243, 303, 336, and 348 mμ. This material was diluted with inactive material and crystallized to constant activity.

7-Methoxy-9-methyl-β-carboline (*ind-N-Methylnorharmine*) (III). *ind*-N-Methylharmine[31] (270 mg.) was dissolved in freshly distilled benzaldehyde (1 ml.) and the mixture refluxed for 3 hr. The excess benzaldehyde was removed *in vacuo* at 75°, and the brown oily residue was dissolved in chloroform and extracted with 5 *N* ammonia to remove benzoic acid. The dried (magnesium sulfate) chloroform layer was evaporated and methanol (2 ml.) added to the residue; benzylidine-*ind*-N-methylharmine separated as light yellow needles (240 mg.), m.p. 156–157°. Additional material (60 mg.) was obtained from the mother liquors and purified by sublimation.

The benzylidine derivative (240 mg.) was dissolved in acetone (25 ml.) and a concentrated aqueous potassium permanganate solution added to the stirred solution at room temperature until no more was consumed. The mixture was filtered through Celite and the filtrate concentrated *in vacuo* to remove acetone. After filtering again the aqueous solution was acidified with hydrochloric acid; the hydrochloride of 7-methoxy-9-methyl-β-carboline-1-carboxylic acid separated out as bright yellow needles, m.p. 210–212°. This salt was dissolved in dilute ammonia and acidified with acetic acid; 7-methoxy-9-methyl-β-carboline-1-carboxylic acid (110 mg.), m.p. 185–186° dec., separated.

Anal. Calcd. for $C_{14}H_{12}O_3N$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.45; H, 4.72; N, 10.67.

(31) F. A. L. Anet, D. Chakracarti, R. Robinson, and E. Schlittler, *J. Chem. Soc.*, 1242 (1954).

The above carboxylic acid (90 mg.) was heated in a sublimation tube at 190° for 5 min. Vacuum was then applied and 7-methoxy-9-methyl-β-carboline sublimed (170° at 0.001 mm.) (70 mg.), m.p. 128–129° (lit.[6b] m.p. 112–114°).

Anal. Calcd. for $C_{13}H_{12}ON_2$: C, 73.56; H, 5.70; N, 13.20. Found: C, 73.70; H, 6.14; N, 13.14.

7-Methoxy-9-methyl-β-carboline 2-Methiodide. The β-carboline III (100 mg.) in methanol (4 ml.) was refluxed with methyl iodide (0.8 ml.). After 15 min. yellow crystals of the methiodide started to separate. More methanol (4 ml.) was added and the refluxing continued for 2.5 hr. Ether (3 ml.) was added and the methiodide (150 mg.) filtered off. Crystallization from ethanol afforded yellow plates, m.p. 279–280°.

Anal. Calcd. for $C_{14}H_{15}ON_2I$: C, 47.47; H, 4.27; N, 7.91. Found: C, 47.71; H, 4.08; N, 7.91.

7-Methoxy-2,9-dimethyl-1,2,3,4-tetrahydro-β-carboline 2-Methiodide (*IV*). The previously described methiodide (130 mg.) was suspended in boiling absolute ethanol (15 ml.) and sodium borohydride (150 mg.) added. After a few minutes the solution became colorless and was allowed to cool to room temperature during 1 hr. The ethanol was removed *in vacuo* and the residue suspended in 1% sodium hydroxide. The tetrahydro-β-carboline derivative was extracted with ether which was then dried (sodium sulfate) and evaporated. The residue was refluxed in methanol (4.5 ml.) with methyl iodide (0.3 ml.) for 2 hr. On concentrating and adding ether the methiodide IV separated (105 mg.), m.p. 212–213°.

Anal. Calcd. for $C_{15}H_{21}ON_2I$: C, 48.39; H, 5.69; N, 7.53. Found: C, 47.91; H, 5.93; N, 7.17.

6-Methoxy-1,2-dimethyl-N,N-dimethyltryptamine Methiodide (*V*). The previous methiodide (IV, 100 mg.) in 15 ml. of liquid ammonia was treated with sodium (20 mg.). After 45 min. additional ammonia (15 ml.) and sodium (5 mg.) were added. After 1 hr., when all the ammonia had evaporated, water was added, followed by ether. Evaporation of the dried (magnesium sulfate) ether extract yielded a residue which was dissolved in methanol (3 ml.) and refluxed with methyl iodide (0.2 ml.) for 2 hr. On cooling, colorless plates of the methiodide V (105 mg.), m.p. 252–253°, separated, identical (mixture melting point, infrared spectrum) with material prepared from 6-methoxy-2-methylindole.

Anal. Calcd. for $C_{16}H_{25}ON_2I$: C, 49.49; H, 6.49; N, 7.22. Found: C, 49.75; H, 6.39; N, 6.91.

This methiodide (100 mg.) was converted to the methohydroxide with silver hydroxide and subjected to a Hofmann degradation using the same procedure as that previously described for an analogous compound.[13] 6-Methoxy-1,2-dimethyl-3-vinylindole (VI) was obtained as a white solid (45 mg.), m.p. 69° (not crystallized). Oxidation of this vinyl compound with osmium tetroxide and then sodium metaperiodate yielded 6-methoxy-1,2-dimethylindole-3-carboxaldehyde (35 mg.), m.p. 130–131°, and formaldehyde collected as its dimedone derivative (30 mg.).

6-Methoxy-2-methylindole-3-carboxaldehyde. 6-Methoxy-2-methylindole[14] (1.61 g.) was formylated with a mixture of dimethylformamide (3.2 g.) and phosphorus oxychloride (1.0 ml.) using the procedure of

Smith.[32] Crystallization of the product from methanol afforded light yellow prisms of the aldehyde (1.85 g.), m.p. 228–229°.

Anal. Calcd. for $C_{11}H_{11}O_2N$: C, 69.82; H, 5.86; N, 7.40. Found: C, 69.43; H, 5.98; N, 7.27.

6-Methoxy-1,2-dimethylindole-3-carboxaldehyde (VII). The previous aldehyde was methylated in aqueous potassium hydroxide with dimethyl sulfate according to previous procedures.[13] Crystallization of the product from benzene yielded long white needles of the aldehyde VII, m.p. 131–132°.

Anal. Calcd. for $C_{12}H_{13}O_2N$: C, 70.91; H, 6.45; N, 6.89. Found: C, 71.21; H, 6.49; N, 6.75.

This material was identical (mixture melting point, infrared spectrum) with material obtained from the degradation of vindoline.

6-Methoxy-2-methyl-3-indoleglyoxylic Acid Amide. Oxalyl chloride (3.25 g.) dissolved in ether (10 ml.) was added slowly at 0° to a stirred solution of 6-methoxy-2-methylindole[14] (4.25 g.) in ether (50 ml.). After stirring for 15 min. the reaction mixture was allowed to warm to 10°. The greenish yellow glyoxylic acid chloride was filtered off and washed with a little ether. This compound was then added with vigorous stirring to concentrated aqueous ammonia (100 ml.). The amide separated as a buff-colored powder (5.12 g.). For analysis some was crystallized from ethanol and had m.p. 268–269°.

Anal. Calcd. for $C_{12}H_{12}O_3N_2$: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.02; H, 5.22; N, 12.03.

6-Methoxy-1,2-dimethyl-N,N-dimethyltryptamine Methiodide. The reduction of the previous amide (2.6 g.) was carried out in a Soxhlet apparatus. The amide was placed in the extraction thimble and the flask contained a solution of lithium aluminum hydride (2.4 g.) in tetrahydrofuran (400 ml.). After 24 hr. almost all the amide had been transferred to the reaction flask. Ether (150 ml.) was then added, followed by water. The reaction mixture was filtered and the filtrate dried over magnesium sulfate. Evaporation yielded 6-methoxy-2-methyltryptamine as a pale yellow oil (1.95 g.). The picrate was obtained as dark brown plates from 95% ethanol, m.p. 212–214°.

Anal. Calcd. for $C_{12}H_{16}ON_2 \cdot C_6H_3O_7N_3$: C, 49.88; H, 4.42; N, 16.16. Found: C, 50.00; H, 4.35; N, 16.09.

This tryptamine was converted to the methiodide V by previously described procedures.[13] The product had m.p. 252–253°.

Deacetylation of Vindoline. Vindoline (350 mg.) was dissolved in 0.5 *N* sulfuric acid (40 ml.) and refluxed for 4 hr. Distillation was then carried out, maintaining the volume in the distillation flask at about 40 ml. by the addition of distilled water. When about 60 ml. of distillate had been collected it was neutralized with 0.1 *N* sodium hydroxide and evaporated to dryness. Recrystallization of the residue from ethanol–ether afforded sodium acetate (36 mg., 57%). Schmidt reaction was carried out on the sodium acetate with sodium azide and concentrated sulfuric acid as previously described.[29] The sodium acetate was converted to 1-acetamidonaphthalene for radioactive assay.

(32) G. F. Smith, *J. Chem. Soc.*, 3842 (1954).

The acidic solution in the distillation flask was made basic with ammonia and extracted with chloroform. The residue obtained on evaporation was found (by thin layer chromatography) to consist of unchanged vindoline and desacetylvindoline. This mixture was dissolved in concentrated hydrochloric acid (5 ml.) and heated on the steam bath for 8 min. The solution was then added to ice, neutralized with ammonia, and extracted with chloroform. The residue obtained on evaporation of the dried (sodium sulfate) chloroform extract was crystallized from ethyl acetate–ether, affording colorless prisms of desacetylvindoline (200 mg.), m.p. 161–162° (lit.[6a] m.p. 156–157°).

Kuhn–Roth Oxidation of Desacetylvindoline. Desacetylvindoline (280 mg.) dissolved in 2 *N* sulfuric acid (10 ml.) was added to a boiling solution of chromium trioxide (8 g.) in 2 *N* sulfuric acid (20 ml.). Distillation was continued until about 100 ml. of distillate had been collected, water being added to maintain the volume in the distillation flask at 20–25 ml. The distillate was neutralized with sodium hydroxide and taken to dryness. Paper chromatography[33] indicated the presence of sodium acetate and propionate. Separation by chromatography on silicic acid[34] afforded sodium acetate (22 mg.) and sodium propionate (7.5 mg.), both assayed by conversion to their α-naphthylamine derivatives.

Kuhn–Roth Oxidation of Catharanthine and Ajmalicine. Catharanthine hemisulfate[6a] [$C_{21}H_{24}O_2N_2 \cdot 0.5(H_2SO_4 \cdot H_2O)$, 150 mg.] oxidized with chromium trioxide (3 g.) in 2 *N* sulfuric acid (30 ml.) yielded a mixture of sodium acetate (5 mg.) and sodium propionate (27.5 mg.).

Ajmalicine (54.4 mg.) was added to a solution of chromium trioxide (1.5 g.) in 2 *N* sulfuric acid (15 ml.) and refluxed for 2 hr. prior to distillation. The yield of sodium acetate (free of sodium propionate) was 12 mg.

Epiibogamine from Catharanthine and Isolation [C-22]. Catharanthine (168 mg.) was dissolved in ethanol (20 ml.) and hydrogenated at 2 atm. for 1 hr. in the presence of platinum oxide (100 mg.). The solution obtained after removal of the catalyst was evaporated to dryness and the residue, dissolved in a little methanol, was added to a solution obtained by dissolving potassium metal (0.5 g.) in a mixture of methanol (5 ml.) and distilled water (1 ml.) which had been boiled to remove carbon dioxide. The solution was refluxed in a nitrogen atmosphere for 6 hr. The solution was then cooled, acidified with 2 *N* sulfuric acid, and boiled for an additional 30 min., the evolved carbon dioxide being collected as barium carbonate (36.2 mg.). In a blank experiment, where the dihydrocatharanthine was omitted, the yield of barium carbonate was less than 1 mg. The contents of the reaction flask was made basic with sodium carbonate and extracted with ether. Thin layer chromatography of the residue obtained on evaporation of the dried ether extract indicated the presence of three compounds, one of them having the same R_f as epiibogamine. The epiibogamine was separated by column chromatography on Woelm alumina (activity III). Fractions obtained on elution with methylene

(33) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, 23, 1033 (1951).
(34) H. F. Mueller, T. E. Larson, and W. J. Lennarz, *ibid.*, 30, 41 (1958).

chloride yielded epiibogamine which was further purified by sublimation *in vacuo* (38 mg.), m.p. 163–165° (lit.[26] m.p. 162–164°).

Acknowledgment. We thank Robert McLeester for the cultivation of the *Vinca rosea* plants in the botany greenhouse of the University of Minnesota.

Reactions in Frozen Systems. II.[1] Enhanced Hydroxylaminolysis of Simple Amides

Harvey E. Alburn and Norman H. Grant

Contribution from the Research Division, Wyeth Laboratories, Radnor, Pennsylvania. *Received March 15, 1965*

Hydroxylaminolysis of the amide bonds of formamide, acetamide, propionamide, glutamine, asparagine, and 2,5-diketopiperazine was studied in ice and in liquid water at several pH values. For acetamide, propionamide, glutamine, asparagine, and 2,5-diketopiperazine, hydroxylaminolysis rates in ice (−18°) exceeded the rates in water (0°) at pH 7 and 8. With glutamine and 2,5-diketopiperazine, the rates at −18° exceeded the rates at 22° at these pH levels. The reaction rate with 2,5-diketopiperazine in −18° frozen solutions exceeded the rate in −11° frozen solutions at pH 6, 7, and 8. The ratios k_1(pH 7)/k_1(pH 6) and k_1(pH 8)/k_1(pH 6) were less than 1.0 in each liquid system but greater than 1.0 in the frozen systems. These results indicate that the phenomenon of rate enhancement in frozen solutions is not limited to ordinarily facile reactions, and the observed differences between liquid and solid systems are inconsistent with a concentration effect in the solids. In addition to mechanisms previously proposed, it is suggested that the dielectric properties of ice may facilitate a concerted attack on the substrate by favoring association of nucleophile molecules.

Introduction

Enhanced rates in ice for the catalytic hydrolysis of the penicillin β-lactam were reported earlier from this laboratory.[1] The evidence did not support the explanation of increased reactant concentrations, and it was suggested that in ice the dielectric properties, the high proton mobility, and the imposition of a favorable orientation of substrate and catalyst might explain the phenomenon. Since this report there have been at least five others showing reaction rate increases in ice.[2–6] Because this is a new area of investigation, it is important to learn whether the reactions which are facilitated in ice are limited to those which occur readily in water. Therefore we extended the study to hydroxylaminolysis of some of the most stable carboxylic acid derivatives, the primary amides and the peptides.

(1) Part I: N. H. Grant, D. E. Clark, and H. E. Alburn, *J. Am. Chem. Soc.*, 83, 4476 (1961).
(2) N. H. Grant, D. E. Clark, and H. E. Alburn, *ibid.*, 84, 876 (1962).
(3) W. H. Prusoff, *Biochim. Biophys. Acta*, 68, 302 (1963).
(4) A. R. Butler and T. C. Bruice, *J. Am. Chem. Soc.*, 86, 313 (1964).
(5) T. C. Bruice and A. R. Butler, *ibid.*, 86, 4104 (1964).
(6) M. W. Weatherburn and J. E. Logan, *Clin. Chim. Acta*, 9, 581 (1964).

Experimental Section

The chemicals used were reagent grade or chromatographically pure. Propionamide was synthesized from the anhydride and recrystallized from benzene. Two lots of hydroxylamine hydrochloride were used: Baker analyzed reagent, assaying 99.5%, was used directly, and Fisher reagent grade, assaying at least 96.5%, was recrystallized from aqueous ethanol. Asparagine was used in the L-form and glutamine in the racemic form.

The reaction mixture contained 0.005, 0.01, or 0.02 *M* substrate, 0.6 *M* hydroxylamine hydrochloride, and sufficient sodium hydroxide to provide a self-buffering system of the desired pH. Solutions at pH 6 and 7 were prepared both with and without the addition of sufficient sodium chloride to equal that finally present in the pH 8 solutions (0.6 *M*). The solutions were distributed into at least six test tubes, stoppered, and set at the appropriate temperatures. For studies at −18°, the solutions were first frozen rapidly in Dry Ice–acetone and then placed in a −18° deep freeze. For −11, 0, and 22° the locations were a thermostated cold room, refrigerator, and laboratory. All samples at 0° remained liquid. Frozen samples were thawed by immersion in a room temperature water bath and then mixed again by holding against a Vortex mixer. The pH after incubation was measured on a Beckman expanded scale pH meter.

For assay, 3-ml. samples were mixed successively with 3 ml. of 0.935 *M* HCl and 1 ml. of 15% ferric ammonium sulfate in 1 *N* H_2SO_4, giving the iron complex whose absorbance was read on a Klett–Summerson spectrophotometer with a 540 mμ filter. In the presence of a large excess of hydroxylamine almost all of the reactions followed clean first-order kinetics for at least two half-lives, and rates were calculated from plots of the first-order equation, $\log (a - x) = -(k_1/2.303)t + \log a$. Determination of the concentration, *a*, of four of the amides was based upon quantitative alkaline hydroxylaminolysis methods.[7,8] In the procedure adopted, 1-ml. samples were incubated with 1 ml. of 1.8 *M* hydroxylamine hydrochloride and 1 ml. of 3.5 *M* sodium hydroxide. Time, temperature, and absorbance for 0.01 *M* amide (from the plot) are as follows: formamide, 6 min., 22°, 1.890; acetamide,

(7) F. Bergmann, *Anal. Chem.*, 24, 1367 (1952).
(8) E. B. Robertson, B. D. Sykes, and H. B. Dunford, *Anal. Biochem.*, 9, 158 (1964).