Table V. Base Sequence of the Model Trinucleotide d-Cl_3CCH_2OpTpApG (6.0 μM)

Step	Yield from oxidative degradation[a] G	A	T
1	3.5	<0.1	<0.1
2	<0.1	2.0	<0.1
3	<0.1	<0.1	1.9

[a] In μmoles of nucleobase.

Table VI. Base Sequence Determination of the Model Dinucleotide d-Cl_3CCH_2OpCpT (10.4 μmol)

Step	Yield from oxidative degradation[a] C	T
1	4.9	<0.15
2	<0.10	<3.0

[a] In μmoles of nucleobase.

Application of the method to a model containing purine bases could now be undertaken. An appropriate model, d-Cl_3CCH_2OpTpApG, synthesized by the procedures of Khorana as modified by Eckstein,[14] was subjected to three cycles of the oxidation–degradation–monophosphorolysis procedures. The results are displayed in Table V. It will be seen that there is never any question of recognizing the proper base signal: any surviving nucleotide is largely protected from subsequent degradation—at the wrong time—presumably by acetylation. The decay of signal strength appears to be greatest at step 1; this seems to be because of the inherent sluggishness of G. Guanosine nucleotides give rise to several products directly upon oxidation; subsequent ammonia hydrolysis to free nucleobase appears to entail some loss. This does not, however, lead to signal ambiguity since no confusing ultraviolet paper spots or spectra were discerned.

Finally, an additional model possessing an internal cytosine was subjected to the analytical procedure since, like d-pG, the yield of nucleobase from its nucleotide reaches a lower yield figure than in the case of the adenine and thymine deoxyribonucleotides. As can be seen from Table VI, unequivocal results were obtained in this case as well.

In summary, it may be stated that the method here described is capable of determining the sequence of model deoxyribooligonucleotides. The limits of its applicability are under study.

Acknowledgment. Thanks are due to Messrs. O. Keller and E. Heimer for the preparation of certain model deoxyribooligonucleotides, and to Dr. C. Harvey for his help with enzymatic procedures.

Biosynthesis of Capsaicin and Dihydrocapsaicin in *Capsicum frutescens*[1]

Edward Leete and Mary C. L. Louden

Contribution from the School of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received May 29, 1968*

Abstract: Capsaicin (N-(4-hydroxy-3-methoxybenzyl)non-*trans*-6-enamide) and 6,7-dihydrocapsaicin are found in approximately equal amounts in *Capsicum frutescens*. These alkaloids, isolated from the fruits of the plant 2 weeks after the administration of DL-phenyl-3-^{14}C-alanine, were labeled solely on the methylene group of their vanillylamine residues. The administration of DL-tyrosine-3-^{14}C afforded alkaloids with very low activity. L-Methionine-methyl-^{14}C afforded radioactive alkaloids, and degradation of the dihydrocapsaicin indicated that all the activity was located on the methoxy group. No activity was found in the alkaloids when DL-mevalonic-2-^{14}C acid or DL-leucine-1-^{14}C were fed to the plant. However, radioactive capsaicin resulted when L-valine-U-^{14}C was fed. Essentially all the activity was located at C-7, -8, -9, and -10 of the 8-methyl-6-nonenic acid moiety of the alkaloid.

Capsaicin (**1**) and its dihydro derivative **2** occur in the fruit of *Capsaicin* species[2] and are responsible for the hot taste of the peppers. In *Capsicum annum* Kosuge and coworkers[3] found capsaicin and its dihydro derivative to be present in the ratio of 7:3. More recently Bennett and Kirby[4] reported the following composition of the phenolic components of *C. annum:* capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%). The presence of the three minor components was established by mass spectrometry.

We commenced work on the biosynthesis of capsaicin in 1965 and have used the plant *C. frutescens* in all our experiments. We found that the phenolic fraction from the fruits of this species contained 47% capsaicin and 53% dihydrocapsaicin. Separation was initially achieved by thin layer chromatography on silica gel plates impregnated with silver nitrate. A more convenient method involved O-methylation, followed by reaction of the mixture with osmium tetroxide. The 6,7-diol **3** derived from capsaicin was readily separated from O-methyldihydrocapsaicin (**4**) by column chroma-

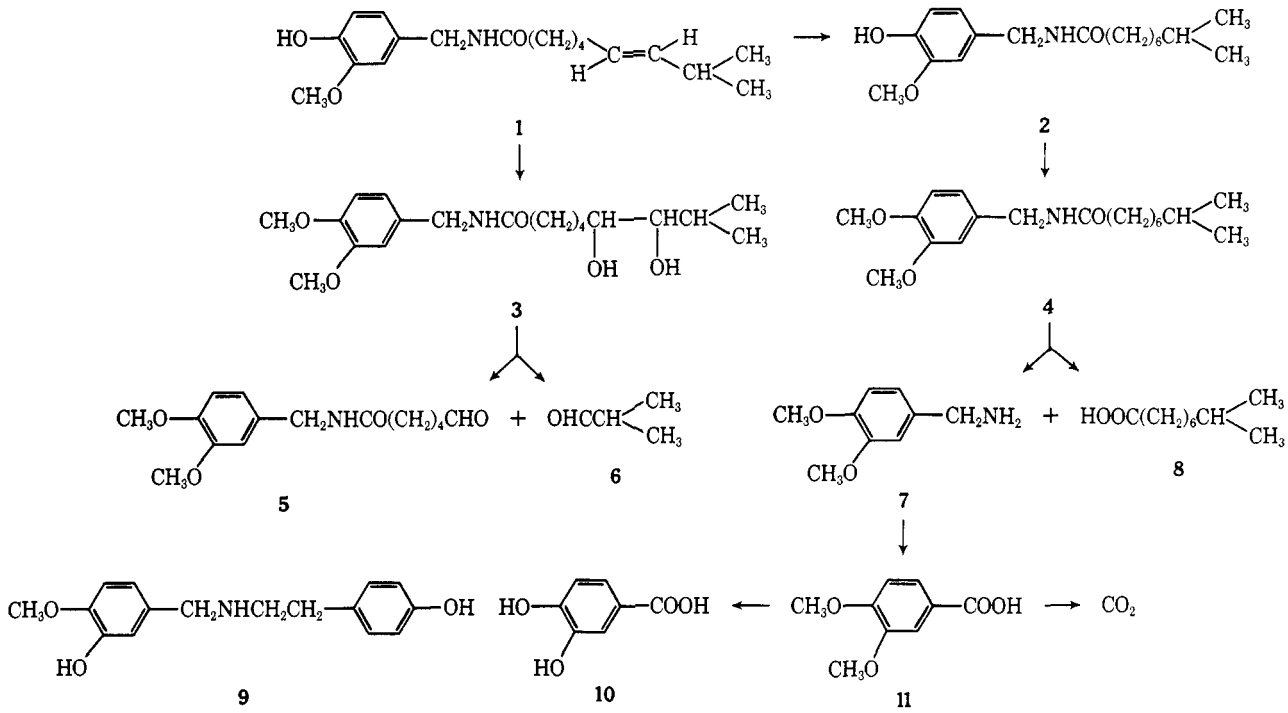
(1) An account of this work was presented at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967. This investigation was supported by a research grant, GB-3696, from the National Science Foundation.

(2) S. Kosuge, Y. Inagaki, and K. Uehara, *Nippon Nogei Kagaku Kaishi*, 32, 578 (1958). These authors assigned the names capsaicin I and II to compounds **1** and **2**, however we prefer the more descriptive names.

(3) S. Kosuge, Y. Inagaki, and H. Okumura, *ibid.*, 35, 923 (1961).

(4) D. J. Bennett and G. W. Kirby, *J. Chem. Soc., C*, 442 (1968).

Scheme I. Degradation of Capsaicin and Dihydrocapsaicin



tography on alumina. We did not attempt to isolate the lower or higher homologs of capsaicin and its dihydro derivative reported by Kirby[4] in *C. annum*. Our crude sample of O-methyldihydrocapsaicin from a typical isolation did apparently contain 4.8% of a higher homolog (detected in its mass spectrum).

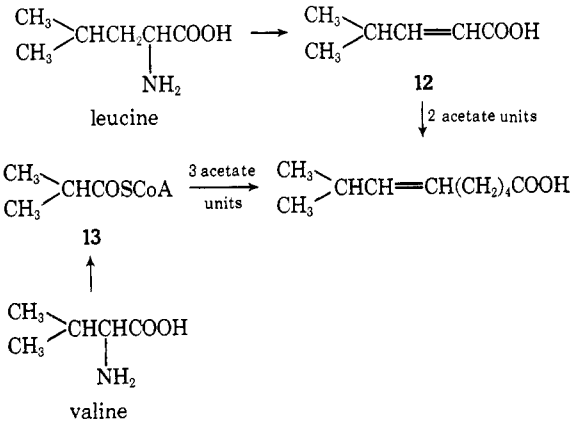
We initially thought that the 4-hydroxy-3-methoxybenzylamine (vanillylamine) moiety of capsaicin would be derived from tyrosine. The formation of vanillylamine would thus be analogous to the formation of gramine from tryptophan.[5] A mechanism for the fragmentation of the tyrosine side chain analogous to the one suggested by Wenkert[6] for the formation of gramine seemed plausible. However, capsaicin and dihydrocapsaicin of very low activity were isolated from *C. frutescens* plants which had been fed DL-tyrosine-3-^{14}C *via* cotton wicks inserted in the stems. We then considered phenylalanine as a precursor since it has been shown that the 3-hydroxy-4-methoxybenzylamine portion of O-methylnorbelladine (**9**) and related *Amaryllidaceae* alkaloids is derived from phenylalanine, but not from tyrosine.[7,8] Indeed the capsaicin and dihydrocapsaicin isolated 2 weeks after the administration of DL-phenyl-3-^{14}C-alanine to *C. frutescens* were radioactive. Degradation of these alkaloids was carried out as illustrated in Scheme I. Dihydrocapsaicin, obtained by hydrogenation of the natural mixture of capsaicin and its dihydro derivative, was O-methylated with dimethyl sulfate in the presence of sodium hydroxide. The resultant O-methyldihydrocapsaicin (**4**) was hydrolyzed with aqueous barium hydroxide at 210°[9] affording veratrylamine (**7**) and 8-methylnonanoic acid (**8**), isolated as its amide. Oxidation of the veratrylamine with potassium permanganate yielded veratric acid (**11**), which was decarboxylated by heating in quinoline in the presence of copper chromite. Essentially all of the activity of the dihydrocapsaicin was located in the resultant carbon dioxide, collected as barium carbonate (see Table II, Experimental Section). Phenylalanine thus served as a direct precursor of the vanillylamine residue of capsaicin and its dihydro derivative. Kirby[4] also found that phenylalanine (tritium labeled) was a precursor of the C_6–C_1 unit of capsaicin. It is probable that the incorporation of phenylalanine into capsaicin proceeds *via* cinnamic acid, *p*-coumaric acid, caffeic acid, and protocatechuic aldehyde as found in other species.[10,11] Kirby has indeed shown that some of these compounds also serve as precursors of capsaicin.[4] The small amount of activity which was found in capsaicin after feeding tyrosine-3-^{14}C or tyrosine-*ar*-3,5-^{3}H[4] could be rationalized by postulating that this species contains a minor amount of the enzyme tyrosine ammonia-lyase, which would catalyze the slow formation of *p*-coumaric acid from tyrosine. Most plant species do not contain a significant amount of tyrosine ammonia-lyase,[12] and the main route to *p*-coumaric acid is from phenylalanine *via* cinnamic acid. The capsaicin derived from tyrosine-3-^{14}C was not degraded because of its low specific activity. It was interesting to discover that a fresh crop of fruits produced 4 months after the initial feeding of phenyl-3-^{14}C-alanine yielded capsaicin and dihydrocapsaicin with essentially the same specific activity as material isolated 2 weeks after feeding. Degradation of the dihydrocapsaicin indicated that there had been no randomization of activity even after this long feeding time. This result suggests that capsaicin and dihydrocapsaicin are stable metabolic end products, unlike several other alkaloids.[13–15] Small but significant

(5) D. O'Donovan and E. Leete, *J. Am. Chem. Soc.*, **85**, 461 (1963).
(6) E. Wenkert, *ibid.*, **84**, 98 (1962).
(7) A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. Wildman, and R. J. Highet, *J. Chem. Soc.*, 1595 (1964).
(8) R. J. Suhadolnik and A. Fischer, *J. Am. Chem. Soc.*, **84**, 4348 (1962).
(9) A. Lapworth and F. A. Royle, *J. Chem. Soc.*, **115**, 1109 (1919).
(10) D. R. McCalla and A. C. Neish, *Can. J. Biochem. Physiol.*, **37**, 537 (1959).
(11) R. J. Suhadolnik, *Abhandl. Deut. Akad. Wiss. Berlin, Kl. Chem. Geol. Biol.*, **3**, 369 (1966).
(12) M. R. Young and A. C. Neish, *Phytochemistry*, **5**, 1121 (1966).

activity was detected in the alkaloids isolated from a third crop of peppers harvested 9 months after the initial feeding of tracer.

We considered that leucine would serve as a precursor of the 8-methylnonenoic acid moiety of capsaicin. A deamination of the amino acid would yield 3-isopropylacrylic acid (**12**). Then extension of this six-carbon acid with two acetate units would yield the required 8-methyl-6-nonenic acid as illustrated in Scheme II.

Scheme II. Hypothesis for the Formation of 8-Methyl-6-nonenic Acid from Leucine and Valine



However the administration of DL-leucine-1-^{14}C to the pepper plants in two separate experiments failed to yield any active alkaloids. We also considered that mevalonic acid might serve as a "starter" for the required branched fatty acid. However, no activity was detected in the alkaloids after feeding mevalonic-2-^{14}C acid to the peppers. The methyl groups found in some branched fatty acids are derived from the methyl group of methionine.[16] The administration of L-methionine-methyl-^{14}C to the peppers yielded radioactive capsaicin and dihydrocapsaicin. However, hydrolysis of the O-methyldihydrocapsaicin yielded 8-methylnonanoic acid which was almost inactive. The veratrylamine resulting from the hydrolysis was radioactive. Oxidation and demethylation afforded dihydroxybenzoic acid which was inactive. The methionine thus served only as a methyl donor for the methoxy group of the alkaloids. Kolattukudy[17,18] has shown that valine is a precursor of the even-numbered iso-branched fatty acids containing 16–26 carbon atoms which are found in tobacco leaves. Kaneda[19] also found that valine-U-^{14}C, but not valine-1-^{14}C yielded radioactive odd-numbered hydrocarbons having a methyl branch C-2. These hydrocarbons are considered to arise by decarboxylation of the corresponding fatty acids containing an even number of carbon atoms. It was suggested[18] that valine is a precursor of isobutyryl coenzyme A (**13**) which serves as a starter for the production of even-numbered iso-fatty acids by reaction with acetate units.

We found that the administration of valine-U-^{14}C to peppers did in fact yield radioactive capsaicin and dihydrocapsaicin. The capsaicin was converted to the diol **3** and cleaved with sodium metaperiodate affording the aldehyde **5** and isobutanal (**6**) which was isolated as its semicarbazone. The latter compound contained essentially all the activity of the capsaicin. A Kuhn–Roth oxidation of the semicarbazone yielded a mixture of isobutyric and acetic acid which were isolated and separated as their α-naphthylamides. The specific activity of the acetic acid derivative was half that of the isobutyryl-α-naphthylamide, a result indicative of uniform labeling of the terminal four carbons of the 8-methyl-6-nonenic acid. Our result is thus consistent with the hypothesis that the ten-carbon acid moiety of capsaicin is formed from isobutyryl coenzyme A and three acetate units. Experiments are in progress with specifically labeled valine and acetate.

Experimental Section

Melting points are corrected. Elementary analyses were carried out by Clark microanalytical laboratory and Mr. J. Chamberlain and his assistants at the University of Minnesota. Infrared spectra were determined on a Perkin-Elmer Model 521 grating spectrophotometer. Mass spectra were determined by Mr. Adrian Swanson on an Hitachi-Perkin-Elmer Model RMU mass spectrometer with a direct inlet system. Radioactive assay was carried out in a Nuclear-Chicago liquid scintillation spectrometer, Model 724, using as solvents either toluene or dioxane with the usual scintillators.[20]

General Method of Feeding the Tracers and Isolation of the Alkaloids. The pepper plants, *Capsicum frutescens*, were grown from seed[21] in a greenhouse, and were usually 3–5 months old at the time of feeding tracers. The phenyl-3-^{14}C-alanine was fed to plants which were 18 months old. The tracers (see Table I for amounts fed and activity and Table II for activities of degradation products) were dissolved in water and administered to the plants *via* cotton wicks which were inserted into the stems of the plants near to ground level. The plants contained a mixture of ripe (red) and unripe (green) fruits and flowers at the time of feeding. The fruits were usually harvested[22] 2–4 weeks after the initial feeding of tracer. The following procedure used for the extraction of peppers which had been fed L-valine-U-^{14}C is typical. The fresh peppers (90 g) were mascerated in a Waring Blendor with a mixture of ether (500 ml) and acetic acid (10 ml). The mixture was filtered and the residue washed with additional ether. The combined ether filtrates were evaporated to small volume, diluted with petroleum ether (bp 60–70°) (300 ml), and extracted with 10% sodium hydroxide solution (five 100-ml portions). The aqueous extract was cooled, acidified with hydrochloric acid, and extracted with chloroform (five 60-ml portions). The dried (Na_2SO_4) chloroform solution was evaporated and the dark brown residue distilled (250°, 0.001 mm) from a Späth distillation bulb, affording a pale yellow semisolid distillate (412 mg). Thin layer plates were prepared from a mixture of Silca Gel G (Merck) (30 g), silver nitrate (1 g), boric acid (1 g), and water (60 ml). A portion of the distillate was subjected to chromatography on these plates, eluting with a 1:1 mixture of chloroform and ethyl acetate. Capsaicin (R_f 0.32) and dihydrocapsaicin (R_f 0.48) were detected by spraying with a solution of potassium permanganate (1%) in 2% sodium carbonate solution (yellow spots on a brown background), or with Millons reagent (reddish brown spots on a colorless background). By the use of thick plates, appreciable quantities of capsaicin and dihydrocapsaicin could be separated. However the usual method of separation involved conversion to their O-methyl ethers followed by treatment with osmium tetroxide. The distilled phenolic fraction (400 mg) was dissolved in 10% sodium hydroxide (50 ml) and shaken with dimethyl sulfate (5 ml) for 30 min. The mixture was extracted with chloroform, dried over sodium sulfate, and then evaporated. The residue was dissolved in ether (100 ml) and osmium tetroxide (300 mg), and two drops of pyridine were added. After standing overnight the dark colored solution was evaporated and the residue

(13) E. Leete and V. M. Bell, *J. Am. Chem. Soc.*, **81**, 4358 (1959).
(14) G. R. Waller, M. S. Tang, M. R. Scott, F. J. Goldberg, J. S. Mayes, and H. Auda, *Plant Physiol.*, **40**, 803 (1965).
(15) G. A. Digenis, B. A. Faraj, and C. I. Abou-Chaar, *Biochem. J.*, **101**, 27C (1966).
(16) *Cf.* J. A. Olson, *Ann. Rev. Biochem.*, **35**, 559 (1966).
(17) P. E. Kolattukudy, *Plant Physiol.*, **43**, 375 (1968).
(18) P. E. Kolattukudy, *Science*, **159**, 498 (1968).
(19) T. Kaneda, *Biochemistry*, **6**, 2023 (1967).
(20) A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141 (1963).
(21) We thank the department of pharmacognosy of the University of Minnesota for a supply of seeds.
(22) The authors acknowledge the help of Peter A. Leete who participated in the tedious picking of the pungent pepper pods.

Table I. Activity of the Compounds Fed to *C. frutescens* and the Resultant Alkaloids

Time of feeding and duration of expt	Compd fed (wt, mg, total act., dpm)	Alkaloids, dpm/mmol (wt isolated,[a] mg) Capsaicin	Dihydrocapsaicin
May 1965	DL-Tyrosine-3-^{14}C[b]	6.1×10^3	6.5×10^3
3 weeks	(2.58, 2.1×10^8)	(33)	(39)
Oct 1966	DL-Phenylalanine-3-^{14}C[c]	1.00×10^5	1.16×10^5
2 weeks	(15.7, 5.2×10^8)	(310)	(356)
4 months		0.83×10^5	1.02×10^5
		(55)	(61)
9 months		0.084×10^5	0.051×10^5
		(60)	(71)
May 1965	DL-Mevalonic-2-^{14}C acid[d]		
3 weeks	(15.1, the DBED[e] salt, 2.07×10^8)	$<1.0 \times 10^3$	$<1.0 \times 10^3$
Dec 1966	DL-Leucine-1-^{14}C[f]		
3 weeks	($8.2, 6.0 \times 10^8$)	$<1.0 \times 10^3$	$<1.0 \times 10^3$
March 1967	DL-Leucine-1-^{14}C	$<1.0 \times 10^3$	$<1.0 \times 10^3$
3 weeks	($8.7, 6.4 \times 10^8$)		
June 1967	L-Methionine-methyl-^{14}C[g]	1.12×10^5	1.14×10^5
3 weeks	($52.2, 5.3 \times 10^7$)	(57.2)	(62.5)
March 1968	L-Valine-U-^{14}C[h]	9.6×10^5	9.5×10^5
4 weeks	($10, 2.2 \times 10^8$)	(32)	(38.5)

[a] The weights isolated are calculated from the O-methyldihydrocapsaicin and the 6,7-diol which were obtained from the chromatographic separation. [b] Purchased from Tracerlab, Waltham, Mass. [c] Purchased from CalBiochem, Los Angeles, Calif. [d] Purchased from New England Nuclear Corp., Boston, Mass. [e] DBED = dibenzylethylenediamine. [f] Purchased from Schwartz Bioresearch Inc., Orangeburg, N. Y. [g] Prepared by reaction of methyl-^{14}C iodide with the sodium salt of L-homocystine: D. B. Melville, J. R. Rachele, and E. B. Keller, *J. Biol. Chem.*, **169**, 419 (1947). [h] Purchased from International Chemical and Nuclear Corp., City of Industry, Calif.

Table II. Activities of the Degradation Products of the Alkaloids

	Activity, dpm/mmol	
	From DL-Phenyl-3-^{14}C-alanine	
	2-week expt[a]	4-month expt[b]
O-Methyldihydrocapsaicin	1.09×10^5	1.02×10^5
Veratrylamine hydrochloride	1.06×10^5	1.02×10^5
Veratric acid	1.07×10^5	1.01×10^5
Barium carbonate	1.03×10^5	0.96×10^5
8-Methylnonamide	0.04×10^5	0.02×10^5
	From L-Methionine-methyl-^{14}C	
O-Methyldihydrocapsaicin	1.14×10^5	
Veratrylamine hydrochloride	1.13×10^5	
Veratric acid	1.11×10^5	
Ethyl 3,4-dihydroxybenzoate	$<0.01 \times 10^5$	
8-Methylnonamide	0.02×10^5	
	From L-Valine-U-^{14}C	
O-Methyl-6,7-dihydroxydihydrocapsaicin	9.6×10^5	
5-Formyl-(3,4-dimethoxybenzyl)pentanamide	0.31×10^5	
Isobutanal semicarbazone	9.2×10^5	
Isobutyryl-α-naphthylamide	9.3×10^5	
Acetyl-α-naphthylamide	4.7×10^5	

[a] This degradation was carried out on the reduced (hydrogen in the presence of Adams catalyst in ethanol as a solvent) mixture of capsaicin and dihydrocapsaicin. [b] This degradation was carried out on the separated O-methyldihydrocapsaicin.

boiled with a mixture of methanol (20 ml), water (10 ml), and sodium sulfite (1 g) for 1 hr. The residue obtained on evaporation of the filtered mixture was suspended in water and extracted with chloroform. Evaporation of the dried (Na_2SO_4) extract afforded a semisolid residue (140 mg), which was dissolved in chloroform and chromatographed on a column of Woelm alumina (activity III) (60 g). O-Methyldihydrocapsaicin was eluted with chloroform, and crystallized from petroleum ether affording colorless plates (40 mg), mp 84–85°. Kirby[4] reported mp 75–76° for material which contained some lower and higher homologs.

Anal. Calcd for $C_{19}H_{31}NO_3$ (321): C, 70.99; H, 9.72; N, 4.36. Found: C, 70.89; H, 9.70; N, 4.31.

The mass spectrum had a molecular ion peak at *m/e* 321. The crude O-methyldihydrocapsaicin obtained directly from the column had a peak at M + 14. Its intensity was 4.8% of the molecular ion peak at 321. Elution of the column with 15% methanol in chloroform afforded O-methyl-6,7-dihydroxydihydrocapsaicin, crystallizing from a mixture of ethyl acetate and petroleum ether as colorless plates (37 mg), mp 88–89°.

Anal. Calcd for $C_{19}H_{31}NO_5$ (353): C, 64.56; H, 8.84; N, 3.96. Found: C, 64.76; H, 9.03; N, 4.19.

The mass spectrum had a molecular ion peak at *m/e* 353, and a pronounced peak at M − 73, presumably due to cleavage of the 6,7-diol.

Degradation of O-Methyldihydrocapsaicin. O-Methyldihydrocapsaicin (100 mg) was heated in a sealed tube with saturated barium hydroxide in water (2.5 ml) at 210° for 18 hr. The contents of the tube were extracted with ether. The filtered ether solution was extracted several times with 2 *N* hydrochloric acid. Evaporation of the acid extract yielded a residue which was crystallized from ethanol, affording colorless needles of veratrylamine hydrochloride (39 mg, 54%), mp 254–255° (lit.[23] 257–258°). The alkaline aqueous solution which had been extracted with ether was acidified with hydrochloric acid and extracted with benzene. The dried (Na_2SO_4) extract was evaporated and thionyl chloride (1 ml) added to the residue. After standing for 3 hr at room temperature, the excess thionyl chloride was removed *in vacuo*, and concentrated ammonia solution (10 ml) added to the residue. After standing for 2 hr the mixture was extracted with chloroform. Evaporation of the

(23) F. Juliusberg, *Ber.*, **40**, 119 (1907).

dried extract yielded a crystalline residue which was crystallized from petroleum ether, affording fine plates of 8-methylnonanamide (27 mg, 50%), mp 102–103°, not depressed on admixture with an authentic specimen, mp 103.5–104.5°.[24]

Degradation of Veratrylamine. Veratrylamine hydrochloride (100 mg) was heated on a steam bath in water (10 ml) containing sodium hydroxide (0.1 g) and potassium permanganate (0.2 g) for 1 hr. The filtered solution was acidified with hydrochloric acid when veratric acid (62 mg), mp 182–183°, separated out. Veratric acid (20 mg) was refluxed in quinoline (1 ml) in the presence of copper chromite (20 mg) in a current of nitrogen. The liberated carbon dioxide was absorbed in barium hydroxide solution yielding barium carbonate (21 mg). Veratric acid (100 mg) was refluxed with 50% hydriodic acid (5 ml) for 24 hr, and then evaporated to dryness. The residue was refluxed with absolute ethanol (5 ml), benzene (5 ml), and a drop of concentrated sulfuric acid for 24 hr. The solution was diluted with more benzene and washed with dilute sodium bicarbonate solution. The organic layer was dried over sodium sulfate and evaporated and the residue sublimed yielding ethyl 3,4-dihydroxybenzoate (21 mg), mp 133–134°, not depressed on admixture with an authentic specimen.

Degradation of O-Methyl-6,7-dihydroxydihydrocapsaicin. Sodium metaperiodate (200 mg) dissolved in water (10 ml) was added to a solution of the diol **3** (100 mg) in methanol (10 ml). After standing for 4 hr at room temperature, water (20 ml) was added and the mixture steam distilled. Semicarbazide hydrochloride (0.5 g) and sodium acetate (1 g) were added to the distillate. After standing overnight the solution was made basic with sodium carbonate and extracted with ether. The dried (Na_2SO_4) extract was evaporated and the residue crystallized from a mixture of benzene and petroleum ether yielding colorless plates of isobutanal semicarbazone (12 mg), mp 122–123°, not depressed on admixture with an authentic specimen. The aqueous solution which had been subjected to steam distillation was extracted with chloroform. The dried extract was evaporated and the residue crystallized from a mixture of ethyl acetate and petroleum ether affording colorless plates of 5-formyl-(3,4-dimethoxybenzyl)pentanamide (63 mg), mp 72–73°.

Anal. Calcd for $C_{15}H_{21}NO_4$ (279): C, 64.49; H, 7.58; N, 5.01. Found: C, 64.38; H, 7.45; N, 5.28.

The mass spectrum had a molecular ion peak at *m/e* 279, and a prominent peak at 251 due to the loss of CO. The infrared spectrum (KBr pellet) contained the following absorptions: 3300 (NH), 2730 (aldehyde CH), 1714 (aldehyde C=O), and 1640 cm^{-1} (amide C=O).

Kuhn–Roth Oxidation of Isobutanal Semicarbazone. The semicarbazone (90 mg) was added to a solution of chromium trioxide (3 g) in 2 *N* sulfuric acid (20 ml) and the mixture refluxed for 12 hr. The mixture was then distilled, water being added to maintain the volume in the distilling flask at about 20 ml. The distillate (50 ml) was titrated with 0.1 *N* sodium hydroxide (3.1 ml required) and evaporated to dryness. The residue (22 mg) was dissolved in water (2 ml), and α-naphthylamine hydrochloride (30 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (100 mg) were added. The resultant mixture of amides was filtered off, washed with dilute acid, and sublimed (140°, 0.01 mm). Thin layer chromatography indicated the presence of the α-naphthylamides of acetic and isobutyric acid. They were separated on preparative tlc plates of Silica Gel F_{254} (Merck), developing with 5% ethanol in chloroform. The zone with R_f 0.45 afforded acetyl-α-naphthylamide (3.5 mg), mp 159–160° (lit.[25] mp 159–160°). The isobutyryl-α-naphthylamide, R_f 0.65 (4.0 mg), had mp 147–148°, obtained as colorless needles from benzene.

Anal. Calcd for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.71; H, 7.10; N, 6.80.

(24) L. F. Fieser, *et al.*, *J. Am. Chem. Soc.*, **70**, 3174 (1948).

(25) E. Leete, H. Gregory, and E. G. Gros, *ibid.*, **87**, 3475 (1965).

Chlorophyll Diastereoisomers. The Nature of Chlorophylls a′ and b′ and Evidence for Bacteriochlorophyll Epimers from Proton Magnetic Resonance Studies[1]

Joseph J. Katz, Gail D. Norman, Walter A. Svec, and Harold H. Strain

Contribution from the Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439. *Received June 17, 1968*

Abstract: Chlorophylls a′ and b′ are shown by pmr to be 10-epichlorophylls a and b. By the use of deuteriochlorophyll a containing ordinary hydrogen at the C-10 position, two kinds of dimer are shown to exist in CCl_4 solution. Bacteriochlorophyll also occurs in diastereoisomeric forms, and pmr shows small amounts of 10-epibacteriochlorophyll to be present in solution.

In 1942, Strain and Manning[2] observed that both chlorophylls a and b were often accompanied by small quantities of a minor green pigment which appeared as a well-resolved, less-sorbed, companion or satellite chromatographic zone. The spectroscopic properties (in the visible region) of the chlorophyll from the a satellite zone were remarkably similar to those of a, and the properties of the other were similar to those of b. The satellite and the principal chlorophyll in each case could be reversibly interconverted by heating in alcohol or pyridine solution, as judged by chromatographic criteria. Consequently, the satellite and the corresponding chlorophyll were considered to be isomers, hence the satellite pigments were designated chlorophyll a′ and b′, respectively. The methyl and ethyl chlorophyllides, which lack the phytyl group, were found to undergo a similar isomerization; hence the reversible isomerization of the chlorophylls must involve the ring system rather than a *cis–trans* isomerization about the vinyl bond in the phytyl moiety. Allomerization products formed from chlorophylls a and b[3] failed to form isomers when heated in propanol solution. Because allomerization is essentially oxida-

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) H. H. Strain and W. M. Manning, *J. Biol. Chem.*, **146**, 275 (1942).

(3) G. R. Seely in "The Chlorophylls," L. P. Vernon and G. R. Seely, Ed., Academic Press, New York, N. Y., 1966, p 91.