from acetone and dried at 60° and 1 mm., m.p. 118.5–119.5°.[7]

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.59. Found: C, 62.59; H, 6.36; N, 14.65.

The analytical material upon hydrolysis yielded a keto acid, m.p. 161–162° after recrystallization from methanol.

Anal. Calcd. for $C_9H_9NO_3$: C, 60.32; H, 5.06; N, 7.82. Found: C, 59.92; H, 4.92; N, 7.85.

All properties of the keto acid corresponded to those of γ-(3-pyridyl)-γ-oxobutyric acid and not to those of a β-oxo compound as previously reported.[1] The keto acid, which did not upon admixture depress the melting point of an authentic sample[6,8] (m.p. 161–163°), was converted to the methyl ester, m.p. 65.5–67.5° (micro). The ester did not depress the melting point of an authentic sample,[8] m.p. 65.5–67.5°, upon admixture and gave a picrate, m.p. 126–128° (micro), which corresponded in turn to an authentic synthetic sample by melting point and mixed melting point.

Anal. Calcd. for $C_{16}H_{14}N_4O_{10}$: C, 45.50; H, 3.34; N, 13.26. Found: C, 45.55; H, 3.36; N, 13.15.

The foregoing keto ester from the metabolic ketoamide (190 mg.) was treated with an excess of hydroxylamine hydrochloride in pyridine and alcohol.[9] A chloroform solution of the resultant oximino ester was chromatographed on Florisil with acetone–benzene to obtain methyl γ-(3-pyridyl)-γ-oximinobutyrate, m.p. 70° (micro).

The oximino methyl ester in chloroform was treated with phosphorus oxychloride essentially in accordance with the previously described conditions[9] for a Beckmann rearrangement of the corresponding oximino ethyl ester. After hydrolysis and alkalinization, the reaction mixture, in contrast to our previous report,[1] contained no 3-pyridylacetic acid. The reaction mixture was extracted with chloroform to obtain 3-aminopyridine which was identified as the picrate, m.p. 199.5–200.5° (micro).

Anal. Calcd. for $C_{11}H_9N_5O_7$: C, 40.87; H, 2.81. Found: C, 40.51; H, 3.10.

The infrared absorption spectra (KBr pellet) of the foregoing and an authentic sample, m.p. 199.5 200.5° (micro), of the picric acid salt showed no essential differences. Upon admixture with the authentic sample, the melting point of the picrate derived from the degradation was not depressed.

The isolation of 3-aminopyridine *via* the Beckmann rearrangement at once pointed to a γ-position for the oxo group in the metabolic ketoamide. Confirmation of this and the demonstration that the structure is actually γ-(3-pyridyl)-γ-oxo-N-methylbutyramide was achieved through a total synthesis of the metabolite. Synthetic methyl γ-(3-pyridyl)-γ-oxobutyrate was treated with an excess of saturated aqueous methylamine overnight at room temperature. The reaction mixture was extracted with chloroform. The residue from evaporation of the chloroform was dissolved in ethyl acetate and treated with decolorizing carbon. The solution deposited crystalline γ - (3 - pyridyl) - γ - oxo - N - methylbutyramide which was recrystallized from benzene to obtain the analytical sample, in low yield, m.p. 119–120°.

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.59. Found: C, 62.59; H, 6.15; N, 14.59.

The sample did not depress the melting point of the metabolic product. The infrared spectra of the natural and synthetic material in chloroform showed no essential difference.[10]

The establishment of the corrected structure of the ketoamide as the methylamide of γ-(3-pyridyl)-γ-oxobutyric acid together with its synthesis provides opportunity for additional studies on the intermediary metabolism of (−)-nicotine.

Details of the foregoing as well as an improved two-step synthesis of γ-(3-pyridyl)-γ-oxo-N-methylbutyramide will be submitted for publication shortly.

(7) This determination was made in a capillary with a heating rate of 0.5°/min., a convenient rate. Slower heating (0.1°/min. after 110°) gave a value of 112.8–113.5°. The compound was found to melt in the same range as the ketoamide obtained[1] previously from dog urine after intravenous administration of (−)-cotinine. Admixture of the two samples of the ketoamide produced no melting point depression. When melted in a capillary, the compound showed some evidence of decomposition, a yellow color, which was more pronounced at lower heating rates. The melt, which showed no tendency to become crystalline, cochromatographed with unmelted material in the ammonia system[6] and showed only one Koenig positive zone. The ketoamide melted at 120–123° on the hot stage (0.5°/min.) and resolidified on cooling.

(8) R. N. Castle and A. Burger, *J. Am. Pharm. Assn. Sci. Ed.*, **43**, 163 (1954).

(9) E. Wada and K. Yamasaki, *J. Am. Chem. Soc.*, **76**, 155 (1954).

(10) The authors are grateful to Mr. J. Scott Osborne, Department of Research and Development, The American Tobacco Company, for the infrared determinations.

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BIOSYNTHESIS IN THE AMARYLLIDACEAE. INCORPORATION OF 3-C^{14}-TYROSINE AND PHENYLALANINE IN *NERINE BOWDENII* W. WATS.[1]

Sir:

Two recent reports have shown that 3-C^{14}-tyrosine is not incorporated into ring A and the benzylic carbon of haemanthamine (I, R = H) in either *Sprekelia formosissima*[2] or *Haemanthus natalensis*,[3] nor is this amino acid a precursor of analogous C_6–C_1 fragments in either haemanthidine (I, R = OH) or tazettine (II) in *S. formosissima*.[2] Because 3-C^{14}-phenylalanine has been reported to be incorporated into ring A and the benzylic carbon of lycorine (III),[4,5] it seemed desirable to examine the

(1) Supported in part by a research grant to Iowa State University of Science and Technology from the National Heart Institute (HE 07503-01).

(2) W. C. Wildman, H. M. Fales and A. R. Battersby, *J. Am. Chem. Soc.*, **84**, 681 (1962).

(3) P. W. Jeffs, *Proc. Chem. Soc.*, 80 (1962).

(4) R. J. Suhadolnik and A. G. Fischer, "Abstracts," Am. Chem. Soc., Chicago, Illinois, 1961, p. 39-Q.

(5) R. J. Suhadolnik, A. G. Fischer and J. Zulalian, *J. Am. Chem. Soc.*, **84**, 4348 (1962).

TABLE I

Fraction	Phenylalanine fed % Incorp.	Phenylalanine fed Dilution[a]	Tyrosine fed % Incorp.	Tyrosine fed Dilution
Chloroform-insoluble alkaloids	0.45		1.02	
Chloroform-soluble alkaloids	0.95		1.42	
Lycorine	0.095	5.46×10^3	0.11	1.55×10^4
Belladine	0.42	1.33×10^3	0.82	3.33×10^3

[a] Specific activity of compound fed (μc./mM.) divided by specific activity of compound isolated.

degradation products are listed in Table II. These data show conclusively that phenylalanine can serve as a precursor of ring A and the benzylic carbon atom in both the lycorine and belladine ring systems but is unable to provide the C_6–C_2 fragment (ring C and the two-carbon side chain) in these alkaloids. In agreement with our earlier results in *S. formosissima*,[2] tyrosine can serve as the precursor of the C_6–C_2 unit, but not the C_6–C_1 unit of belladine.

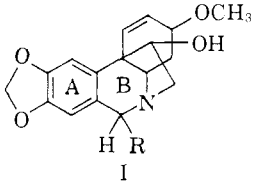
Superficially, these findings are in conflict with the fact that in most Amaryllidaceae alkaloids ring

TABLE II[a]

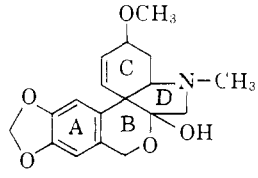
Fragment	Isolated and counted as	Relative activity Phenylalanine	Relative activity Tyrosine
IV	Belladine (IV)	1.00	1.00
IV	Belladine methiodide	0.96	0.95
IV, Ring A + C_1'	N,N,N-Trimethylveratrylammonium iodide	.92	.00
IV, Ring C + C_1, C_2	1-(*p*-Methoxyphenyl)-1,2-dibromoethane	.01	.99
IV, Ring C + C_1, C_2	1-(*p*-Methoxyphenyl)-1,2-ethanediol	.01	1.00
IV, C_1	Formaldehyde (methone)	.00	0.00
IV, Ring C + C_2	Anisaldehyde (octahydroxanthene)	.01	0.98
III	Lycorine	1.00	..
III	4-(1,2-Dihydroxyethyl)-5,6-dihydro-5-methyl-8,9-methylenedioxyphenanthridine	0.99	..
III, C_5	Formaldehyde (methone)	0.00	..
III, less C_5	4-Carboxy-5-methyl-8,9-methylenedioxyphenanthridinone	1.02	..
III, C_4	Barium carbonate	0.00	..
III, less C_4, C_5	5-Methyl-8,9-methylenedioxyphenanthridinone	1.04	..
III, less C_4, C_5	5-Methyl-8,9-methylenedioxy-6-phenylphenanthridinium perchlorate	0.95	..
III, C_7	Benzoic acid	0.97	..

[a] Samples were counted in a Packard Tri-carb Scintillation Counter in toluene or dioxane–naphthalene scintillator solutions.

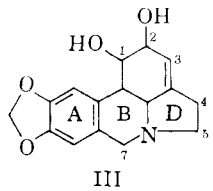
incorporation of these amino acids in *Nerine bowdenii*, where at least two alkaloid ring systems could be examined simultaneously.



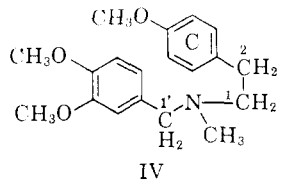
I



II



III



IV

Solutions of the hydrochlorides of 3-C^{14}-DL-phenylalanine (0.3 mc.) and 3-C^{14}-DL-tyrosine (0.1 mc.) were injected into the bulbs of blooming *N. bowdenii*. After one month the bulbs were harvested and processed in the usual way.[6] Total incorporation of radioactivity of phenylalanine and tyrosine into the various alkaloid fractions is given in Table I. The lycorine (III) and belladine (IV), after appropriate dilution with inactive alkaloids, were degraded by the methods described in a previous paper.[6] The relative specific activities of pertinent

(6) W. C. Wildman, H. M. Fales, R. J. Highet, S. W. Breuer and A. R. Battersby, *Proc. Chem. Soc.*, 180 (1962).

A possesses a greater degree of hydroxylation than ring C. However, similar findings have been reported in the biosynthesis of the phenolic cinnamic acids related to lignin.[7] A more detailed study of the processes by which oxygen is introduced into the C_6–C_1 unit of these alkaloids is in progress.

(7) D. R. McCalla and A. C. Neish, *Can. J. Biochem. and Physiol.*, **37**, 537 (1959).

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DOUBLE ADDITION OF A CARBENE TO AN ACETYLENE

Sir:

Hydrocarbon bicyclobutanes have recently been prepared by double carbene sequences.[1,2] We wish to report the formation of a perfluorobicyclobutane by adding difluorocarbene twice to hexafluoro-2-butyne.

Difluorocarbene was generated by pyrolysis of $(CF_3)_3PF_2$ at 100°[3] and added to hexafluoro-2-butyne in the gas phase to give 1,2-bis-(trifluoro-

(1) R. Breslow, Organic Symposium, Bloomington, Indiana, June, 1961.

(2) W. R. Moore, H. R. Ward and R. F. Merritt, *J. Am. Chem. Soc.*, **83**, 2019 (1961).

(3) W. Mahler, Abstracts, 2nd International Symposium on Fluorine Chemistry, Estes Park, Colo., 1962, p. 90.