the 1.53–1.57 μ position characteristic of the amide–amide hydrogen bond to another wave length characteristic of the amide–acid interaction.

The spectra of poly-γ-benzyl-L-glutamate in the "helix-forming" solvents, chloroform and pyridine, did indeed reveal double absorption bands at 1.53–1.57 μ with extinction coefficients almost identical with those of the model amide in the amide hydrogen bonded form. Thus, the spectral properties of this interaction are not markedly changed when the C=O and the NH groups are part of a polypeptide structure. Spectra of solutions of poly-γ-benzyl-L-glutamate in dichloroacetic acid, as well as those of solutions of poly-L-alanine in dichloroacetic acid and in trifluoroacetic acid and of poly-L-leucine in trifluoroacetic acid, exhibited only a single peak which, however, appeared in the neighborhood of 1.51 μ, a band position hitherto unobserved in the spectra of the model amide. The spectral characteristics of the polymers in these organic acids are summarized in Table I. A spectrum of poly-L-alanine is shown in Fig. 1-A.

TABLE I

SPECTRAL CHARACTERISTICS OF POLYAMINO ACIDS IN "HELIX-BREAKING" SOLVENTS

Polymer	Solvent	Position of absorption maximum, μ	Extinction coefficient, cm.²/mole amino acid residue
Poly-γ-benzyl-L-glutamate	$CHCl_2CO_2H$	1.510[a]	165[a]
Poly-L-alanine	$CHCl_2CO_2H$	1.510	230
	CF_3CO_2H	1.505	200
Poly-L-leucine	CF_3CO_2H	1.508	172

[a] Spectrum recorded on a Beckman DK-2 spectrophotometer; all others taken on a Cary Model 14 CMR.

To identify the structural state of the peptide corresponding to this 1.51 μ band, we have returned to our model compound, N-methylacetamide. Dissolved in trifluoroacetic acid, this amide (read against a reference solution containing N,N-dimethylacetamide) exhibited a spectrum with a double-humped band between 1.510 and 1.525 μ, as shown in Fig. 1-A. Despite the presence of an extra peak at 1.525 μ in this model amide, the following experimental evidence indicates that both N-methylacetamide and poly-L-alanine are protonated in trifluoroacetic acid.

(1) Protonation of N-methylacetamide in mixed solvents containing perchloric acid, dioxane, and water has been followed by the technique of nuclear magnetic resonance.[7] Under conditions where these data indicate protonation of the amide (predominantly on the oxygen[7]), the near-infrared spectra of N-methylacetamide (read against reference solutions containing N,N-dimethylacetamide) reveal an NH peak at 1.51 μ. This same peak is also exhibited in this perchloric acid solvent when the amide group is incorporated in a polypeptide structure such as poly-L-alanine (see Fig. 1-B). Solutions of N-methylacetamide in other strong acids, sulfuric and hydrochloric, also exhibit maxima at 1.51 μ. The presence of this band in solutions of hydrochloric acid removes any objection that such peaks may reflect specific hydrogen bonding between the amide groups and the oxygens of $HClO_4$, H_2SO_4 or CF_3COOH. Spectra of the amide in these strong acids are shown together in Fig. 1-C.

(2) Measurements of the apparent specific volumes (Table II) of both the low molecular weight amides, N-methylacetamide and N,N-dimethylacetamide, and the polymer, poly-L-alanine, in trifluoroacetic acid indicate a pronounced contraction in the volume of these solutions. This contraction is symptomatic of electrostriction, which would be produced by charged groups created in the protonation reaction.

(7) A. Berger, A. Loewenstein and S. Meiboom, *J. Am. Chem. Soc.*, **81**, 62 (1959).

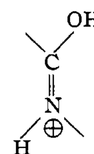
TABLE II

APPARENT SPECIFIC VOLUMES OF MODEL AMIDES AND POLY-L-ALANINE

Solute	Solvent	Concentration, moles/l.	Apparent specific volume, ml./g.
N-Methylacetamide	CF_3CO_2H	2.00	0.700
		.309	.451
		.274	.433
		0	.31 (extrap.)
	CCl_4	0	1.02 (extrap.)
	Pure liquid		1.06
N,N-Dimethylacetamide	CF_3CO_2H	1.00	0.62
		.446	.554
		.270	.498
		0	.35 (extrap.)
	Pure liquid		1.07
Poly-L-alanine	CF_3CO_2H	0.849[a]	0.65
	Amino acid residue		.74[b]

[a] Concentration in moles of amino acid residue/l. [b] Taken from E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold Publishing Co., New York, N. Y., 1943, p. 372.

It may thus be concluded that a peak in the neighborhood of 1.51 μ is diagnostic of the protonated amide form



and that this form characterizes the state of the amide groups of polypeptides in trifluoroacetic and dichloroacetic acids.

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(9) Postdoctorate Fellow of the U. S. Public Health Service.

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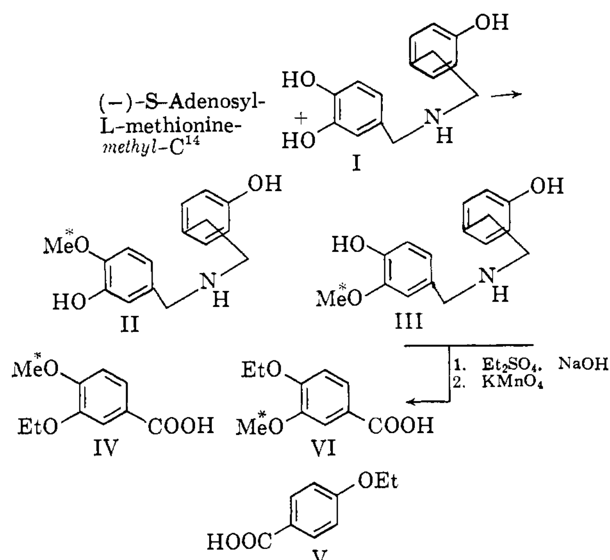
In Vitro Alkaloid Biosynthesis in the Amaryllidaceae; Norbelladine O-Methylpherase

Sir:

Radioactive norbelladine (I) is incorporated without degradation into alkaloids such as belladine, galanthamine, crinamine, and lycorine by intact plants of the Amaryllidaceae.[1] However, this approach requires long periods of time and yields are usually low. We have sought to avoid these difficulties by employing partially purified cell-free enzyme systems.

Flowering bulbs of *Nerine bowdenii* were homogenized in a 0.05 *M* Tris buffer (pH 7.4) at 4°, filtered, and centrifuged at 5000*g*. Ammonium sulfate precipitation and dialysis or Sephadex treatment yielded a partially purified preparation which catalyzed the O-methylation of norbelladine (I) and of other catechols. The preferred methyl donor is (−)-S-adenosyl-L-methionine; diastereoisomers of this compound are less active, and such potential methyl donors as S-methylmethionine, betaine, methionine sulfoxide,

(1) Most recent paper: W. C. Wildman, A. R. Battersby and S. W. Breuer, *J. Am. Chem. Soc.*, **84**, 4599 (1962).



methionine sulfone, and choline are without effect. Monomethylated derivatives II and III are not further methylated.

Norbelladine (I, 250 mμmoles) was incubated at pH 8.1 for 5 hr. at 26° with (−)-S-adenosylmethionine-*methyl*-C^{14} (150 mμmoles, specific activity, 9.2 c./mole) in the presence of *Nerine* enzyme (4.3 mg. of protein). Radioactive material was then extracted into ethyl acetate by adjusting the reaction mixture to pH 10. Separate portions of this extract were used to determine the extent of *para* and *meta*-O-methylation of the catechol moiety of norbelladine (I).

Synthetic N-isovanillyltyramine (II)[2] (m.p. 183–185°; *Anal.* Found: C, 70.09; H, 7.03) was added to one aliquot; 78% of the radioactivity could not be separated from this compound upon repeated crystallization from ethanol. To rule out possible contamination with isomer III, the recrystallized material was degraded *via* O-ethylation with diethyl sulfate and alkali followed by oxidation with potassium permanganate. The resulting acids were converted to their methyl esters with diazomethane and subjected to preparative gas chromatography. This procedure separated the non-radioactive ester of acid V (acid m.p. 195–196°[3]) from a fraction containing a possible mixture of the esters of acids IV and VI. The latter fraction was hydrolyzed and acid IV, m.p. 165–166°,[4] was freed from any trace of acid VI, m.p. 193–194°[5] (see below) by repeated recrystallization to the same constant molar specific radioactivity as phenol II.

To determine the amount of *meta*-methylation, N-vanillyltyramine hydrobromide (III-hydrobromide, m.p. 158–162°; *Anal.* Found: C, 54.09; H, 5.69) was added to another aliquot of the radioactive extract. This material could not be recrystallized to constant specific activity, since isomers II and III (as hydrobromide) co-crystallized. Accordingly, this mixture was degraded as described above. Acid VI was freed from traces of radioactive acid IV by repeated recrystallization and finally retained only 3.5% of the original radioactivity of the extract.

These results indicate that the ratio of *para* to *meta*-methylation of norbelladine catalyzed by the *Nerine* enzyme is 22:1. Approximately 4% of the initial norbelladine had been methylated, although no attempt was made to obtain complete consumption of either substrate.

Another enzyme, present in rat liver, also catalyzes the methylation of catechols using (−)-S-adenosyl-L-methionine as a methyl donor.[6] The products of this enzyme have been shown to be predominantly *meta*-O-methylated isomers.[7] Rat liver enzyme (4.5 mg. of protein) was incubated for 4 hr. at 37° under conditions similar to those used for the plant enzyme, but in the presence of 5×10^{-4} *M* magnesium chloride. The *para* to *meta* ratio (II:III) was found to be 0.28:1, indicating that the highly specific *para*-methylation is a property of the plant enzyme. Approximately 18% of the norbelladine had been methylated. Barton, Kirby and Taylor have shown recently[8] that the *para*-O-methylated phenol II was converted into haemanthamine without loss of the methoxyl carbon, *i.e.*, cyclization of the methoxyl to a methylenedioxy group had occurred. The *meta*-O-methylated isomer III apparently was not tested. The results reported herein suggest that the main biosynthetic route from norbelladine to haemanthamine proceeds by way of II rather than III. The findings suggest also that (−)-S-adenosyl-L-methionine is the source of the methylenedioxy groups in the Amaryllidaceae alkaloids, and are in accord with the earlier observation by Scribney and Kirkwood,[9] who found that the methyl carbon of L-methionine was a precursor of the methylenedioxy group of the Papaveraceae alkaloid protopine.

(2) D. H. R. Barton, G. W. Kirby, J. B. Taylor and G. M. Thomas, *Proc. Chem. Soc.*, 254 (1961). Professor A. R. Battersby has informed us that he and S. W. Breuer have recently established the presence of II in double Narcissus plants (private communication).

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(4) E. Spath and E. Bernhauer, *Ber.*, **58**, 203 (1925).

(5) F. Tiemann, *ibid.*, **8**, 1130 (1875).

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(9) M. Scribney and S. Kirkwood, *Nature*, **171**, 931 (1953).

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The Synthesis of 3-β-D-Ribofuranosyladenine[1]

Sir:

Reports of the isolation of naturally occurring 3-substituted purines, triacanthine (6-amino-3-(γ,γ-dimethylallyl)-purine)[2–4] and 3-ribosyluric acid,[5] stimulated us to synthesize the isomer of adenosine, 3-ribofuranosyladenine (Ia), in order to compare its behavior in chemical and biological systems with that of adenosine, for example, to see whether adenosine and "3-isoadenosine" exhibit a relationship similar to that of uridine and pseudouridine.[6]

Direct alkylation on the 3-position of adenine has recently been shown to be not only a possibility but a preference,[2,7–9] and we are now able to describe a

(1) Supported in part by a Research Grant (USPHS-RG5829, currently GM-05829-05) from the National Institutes of Health, U. S. Public Health Service.

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(8) B. C. Pal, *Biochemistry*, **1**, 558 (1962).

(9) 3-Allylation and 3-benzylation by T. Fujii, University of Illinois, reported by N. J. Leonard at The International Symposium on Organic