teins and polypeptides,[5] the rate of increase in levorotation was compared with the rate of enzyme inactivation and found to be indistinguishable.

TABLE I

THE KINETICS OF PEPSIN INACTIVATION BY FOUR DIFFERENT METHODS

All solutions were adjusted from unbuffered stock solutions, which were near *p*H 5.5, to their experimental *p*H values by the addition of relatively small volumes of concentrated buffers. The *p*H of the buffers were only slightly higher than the *p*H of the experiment. The pepsin concentration is for the total weight of sample. These crystalline preparations of pepsin (Worthington Biochemical Corporation) contain about 20% split products. The light scattering experiments were performed at room temperature, which was 28°; in the other procedures the temperature was controlled at 25.0°. An Ostwald viscometer was used with a flow time of 89.2 sec. The optical rotatory change was for a 2-dm. cell. Tris buffer is tris-(hydroxymethyl)-aminomethane.

	Method	Pepsin g./100 ml.	*p*H	Buffer	*M*	Net observed change	$k \times 10^2$ (min.$^{-1}$)
1	Light scatter	0.35	6.42	Cacodylic KNO_3	0.023 0.136	−60%	4.4
2	Viscosity	0.33	7.10	Imidazole NaCl	0.015 0.009	+3.1 sec.	4.8
3	Optical rotation	0.80	7.08	Imidazole KNO_3	0.045 0.008	−0.25°	5.7
4	Acid liberation	0.42	6.64	Tris NaCl	0.023 0.146	5.6 H/ mole pepsin)	3.4

Finally in the conversion of pepsin from a catalytically active protein to an inactive form (in the *p*H range 6.64–6.97), about 5.6 moles of hydrogen ions are liberated per mole of enzyme. In four experiments in this *p*H interval, the first order velocity constants agreed with the rate of inactivation[6] within 5%. The unmasking of these acidic groups is reflected by appreciable differences in the titration curves of native and denatured pepsin above *p*H 6. These groups are displaced to lower *p*H values in the denatured form.

The acid formed in the inactivation of pepsin can be most readily accounted for if it comes from hydrogen-bonded carboxyl groups. The intrinsic *pK* of carboxyl groups in a number of proteins is near 4.5.[7] The *pK* of these groups in pepsin are increased from their intrinsic values both by a sizable contribution from the electrostatic free energy[8] and by the free energy of hydrogen bonding.[9] It should be noted that part of the 5.6 moles of acid formed probably comes from non-hydrogen bonded ionizable groups since the change in the mass and shape of pepsin decreases the electrostatic free energy of ionization.[10]

It thus appears that the loss of pepsin enzyme activity is accompanied by the appearance of both hydrophilic and hydrophobic groups, gross changes in the mass and shape of its molecular kinetic unit and that the bio- and physico-chemical alterations are expressions of a single rate-determining process, which manifestly involves the rupture of carboxyl linked hydrogen bonds.

(5) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 499 (1956); R. B. Simpson and W. Kauzmann, *ibid.*, **75**, 5139 (1953).

(6) M. L. Anson, *J. Gen. Physiol.*, **22**, 79 (1938). The test was modified to the extent that the concentrations of split products were determined by their absorption at 280 mμ.

(7) C. Tanford, S. A. Swanson and W. S. Shore, THIS JOURNAL, **77**, 6414 (1955), *cf.* Table III.

(8) G. E. Perlmann, in "Advances in Protein Chemistry," M. L. Anson, K. Bailey and J. T. Edsall, Vol. X, Academic Press, Inc., New York, N. Y., has reported that the isoelectric point increases from a value near *p*H 1.0 to 1.7 when the single phosphate group of pepsin is removed by enzymatic means.

(9) M. Laskowski, Jr., and H. A. Scheraga, THIS JOURNAL, **76**, 6305 (1954).

(10) As the NaCl concentration is increased to 1*M* the acid liberated decreases to about 75% of its value in 0.15*M* NaCl. This would leave about 4 moles of acid originating from hydrogen-bonded groups.

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BIOSYNTHESIS OF THE PYRIDINE RING OF NICOTINE[1]

Sir:

A biosynthetic connection between nicotinic acid, or its supposed antecedents, and the pyridine ring of nicotine has been proposed.[2,3,4] However, isotopic tracer experiments using carboxyl-C^{14} nicotinic[5] and anthranilic[6] acids and tryptophan-β-C^{14}[7] have failed to yield supporting evidence. Recently, Dewey, Byerrum and Ball[8] and Leete[9] reported that the pyrrolidine ring of nicotine, and in particular the carbon atom at position 2′ in the pyrrolidine ring, is derived from ornithine. If this is correct, obviously the labeled atoms of the three compounds listed could not be retained in the newly formed nicotine molecule. Therefore, the experiments cited above[5,6,7] are inapplicable.

Ring-labeled H^3-nicotinic acid was prepared by neutron irradiation of a mixture of the acid and lithium carbonate,[10] with carboxyl tritium being removed during the purification of the acid. Nicotinic acid containing C^{14} in both ring and carboxyl positions was obtained by neutron irradiation of nicotinamide.[11]

We have supplied these two preparations to *sterile* cultures of excised roots of Turkish tobacco[5] and have found substantial amounts of radioactivity in the nicotine produced by the roots during their growth (Table I). Lesser incorporation of C^{14} label was partly due to the use of limiting amounts of nicotinic acid, and also to the loss of nicotinic acid carboxyl carbon.[5] Even in this case, however,

(1) Work performed under the auspices of the U. S. Atomic Energy Commission at Brookhaven National Laboratory and at Columbia University under Contract No. AT(30-1)-1778.

(2) E. Winterstein and G. Trier, "Die Alkaloide," 2nd ed., Borntraeger, Berlin, 1931, p. 1031.

(3) G. Klein and H. Linser, *Planta*, **20**, 470 (1933).

(4) R. Dawson, *Plant Physiol.*, **14**, 479 (1939).

(5) R. Dawson, D. Christman and R. C. Anderson, THIS JOURNAL, **75**, 5114 (1953).

(6) R. Dawson and D. Christman, unpublished data.

(7) K. Bowden, *Nature*, **172**, 768 (1953).

(8) L. Dewey, R. Byerrum and C. Ball, *Biochem. et Biophys. Acta*, **18**, 141 (1955).

(9) E. Leete, *Chem. and Ind.*, No. **19**, 537 (1955).

(10) R. Wolfgang, F. Rowland and C. Turton, *Science*, **121**, 715 (1955).

(11) A. Wolf and R. C. Anderson, THIS JOURNAL, **77**, 1609 (1955); *cf.* R. C. Anderson, E. Penna-Franca and A. Wolf, Brookhaven National Laboratory Quarterly Progress Report, October 1–December 31, 1954.

a quite significant amount of radioactivity appeared in the nicotine.

TABLE I

Compound supplied	Acid specific activity (d.p.m./mg. C or H)	Nicotine yield, mg.	Nicotine specific activity (d.p.m./mg. C or H)
1 75 mg. nicotinic acid-H^3	6.4×10^6	25	1.73×10^6
2 44 mg. nicotinic acid-H^3	6.4×10^6	25	1.33×10^6
3 9 mg. nicotinic acid-C^{14}	1910	24	102

Oxidation of the tritium-labeled nicotine (sample 1, Table I) with hot concentrated nitric acid yielded nicotinic acid having a specific activity of 2.97×10^6 d.p.m./mg. H. Owing to the possibility that isotope exchange might have occurred under such drastic conditions, the oxidation was repeated with aqueous potassium permanganate as reagent. Nicotinic acid was obtained which had a specific activity of 4.74×10^6 d.p.m./mg. H. This can be compared to the activity of the nicotine (sample 1, Table I), which is 4.84×10^6 d.p.m./mg. H *as nicotinic acid* (assuming the pyrrolidine hydrogens to be inactive), and thus represents an almost complete recovery of tritium from the nicotine.

Further details of these and other supporting experiments will appear in a later paper.

It is thus probable that the pyridine ring of nicotinic acid is a biosynthetic precursor of the pyridine ring of nicotine and of nornicotine[12] and perhaps also of the related tobacco alkaloids, myosmine, anabasine, anatabine, etc. It is of interest to note that this work, together with that of Dewey, Byerrum and Ball[8] and Leete,[9] constitutes the first recorded biosynthetic pathway for a plant alkaloid. It is further noteworthy that a universally distributed cofactor of biological catalysts and an important amino acid metabolite should be involved as intermediates in the syntheses of one of a class of substances for which no biochemical or physiological significance has yet been adduced.

(12) R. Dawson, THIS JOURNAL, **67**, 503 (1945).

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AN ASYMMETRIC COPOLYMER SYNTHESIS

Sir:

The multiple possibilities for stereoisomerism in vinyl polymers containing monosubstituted mers was early used by Staudinger[1] to explain their generally amorphous character and by Huggins[2] to explain the dependence of their physical properties on temperature of polymerization.[3]

Practical methods have now been devised to prepare more crystalline monosubstituted vinyl polymers, which are said to contain an alternating sequence of D and L configurations[4] and others apparently containing sequences of adjacent mers of like configuration.[5,6]

The closely related problem of preparing a polymer or copolymer with an excess of one configuration in the backbone of the polymer chain and exhibiting optical activity was first attacked unsuccessfully about sixty years ago.[7] Since then further attempts have been made by initiating polymerization with optically active acyl peroxides[8] and by polymerizing and copolymerizing optically active monomers.[9,10] Although the latter method might be expected to induce an excess of one configuration during polymerization, removal of the optically active centers initially present has so far left inactive polymers.

In the following synthesis we have found evidence of induced asymmetry during vinyl polymerization: 1-α-methylbenzyl alcohol (I) ($(M)^{25}D$ $-41.5°$, $a = -17.0°$, $l = 0.5$ cm.)[11] was allowed to react with methacrylyl chloride[12] in pyridine. *l*-α-Methylbenzyl methacrylate (II) (b.p. 92° at 3–4 mm.) was isolated by conventional methods in 65% yield. (*Anal.* Calculated for $C_{12}H_{14}O_2$: C, 75.78; H, 7.4. Found: C, 75.8; H, 7.7. $[M]^{25}D$ $-78.8°$, $a = -20.74°$; $l = 0.5$ dcm.).

(II) was polymerized in the absence of air in peroxide-free dioxane at 35° for 32 hours or more. α,α'-Azobisisobutyronitrile (ABIN) photosensitized by irradiation from a Type AH4 Hanovia Mercury lamp (General Electric Co.) was used as initiator.[13] The polymer (III) isolated by precipitations in petroleum ether and freeze drying in dioxane was found to have an ultraviolet absorption spectrum with peaks at 252, 258 and 264 mμ, characteristic of the α-methylbenzyl group, and a negative rotation. ($[M]^{25}D$ -147, $a = -0.87$, $l = 0.5$ dcm., $c = 2.2\%$ in dioxane.) Calcd. for $(C_{12}H_{14}O_2)_n$: C, 75.7; H, 7.4. Found: C, 75.2; H, 7.7.

The α-methylbenzyl groups were removed by heating 0.300 g. of (III) with 2 g. of phosphonium iodide (PH_4I) in acetic acid at 60°.[14]

The peaks of the ultraviolet absorption spectrum of the reduced polymer disappeared into the background absorption on reduction; the analysis indicated complete removal of the ester groups (calcd. for $(C_4H_6O_2)_n$: C, 55.9; H, 7.0. Found: C, 55.7; H, 7.5) and as expected[15] the resulting polymer (IV) showed no optical activity when observed under the same conditions as above.

When (II) was similarly copolymerized with maleic anhydride in a 1:3 molar ratio of ester to maleic

(1) H. Staudinger, "Die Hochmolekularen organische Verbindungen," Julius Springer, Berlin, 1932, p. 114.
(2) M. L. Huggins, THIS JOURNAL, **66**, 1991 (1944).
(3) T. Alfrey, A. Bartovics and H. Mark, *ibid.*, **66**, 2319 (1943).
(4) C. E. Schildknecht, *et al.*, *Ind. Eng. Chem.*, **40**, 2104 (1948); **41**, 1998 (1949); **41**, 2391 (1949).
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