The dark, tarry residue from the distillation was extracted with 200 ml. of 95% ethanol, decolorized with carbon, filtered, and concentrated to one-half the original volume. After standing overnight in a refrigerator, there was obtained 22.5 g. (28.4%) of 3-butyl-3-propyl-1-octyl 3,5-dinitrobenzoate (IV) in the form of yellowish crystals, m.p. 42–45°. An analytical sample was obtained from 95% ethanol as fluffy, colorless needles, m.p. 44.5–45°.

Anal. Calcd. for $C_{22}H_{34}O_6N_2$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.60; H, 8.03; N, 6.56.

Saponification of IV. In a 500-ml. three necked flask equipped with a mechanical stirrer and a reflux condenser were placed 20.3 g. (.31 mole) of 85% potassium hydroxide pellets, 200 ml. of 95% ethanol, and 50 ml. of water, and the mixture was stirred until the potassium hydroxide went into solution. To the alkali solution was added 15.4 g. (.037 mole) of IV and the mixture was refluxed for 25 hr. It was allowed to cool and 1-l. of water was added. The mixture was extracted four times with ether, the ether removed, and the residue distilled under reduced pressure. There was obtained 7.1 g. (84.5%) of II as a viscous, colorless oil, b.p. 131–132° (1.5 mm.), n_D^{25} 1.4517.

Anal. Calcd. for $C_{15}H_{32}O$: C, 78.87; H, 14.12. Found: C, 78.85; H, 13.82.

Identification of III. A solution of 20 g. (.30 mole) of 85% potassium hydroxide in 50 ml. of water and 100 ml. of 95% ethanol was refluxed with 10.9 g. of III for 36 hr., cooled, and transferred to a distillation flask. The ethanol was removed by distillation, and after adding 150 ml. of water, the mixture was extracted four times with ether. The combined ether extracts were washed with water, dried over magnesium sulfate, filtered, and the ether removed. The residue was distilled under reduced pressure and 7.8 g. of a colorless, viscous oil, b.p. 139–140° (2.0 mm.), n_D^{25} 1.4519, was obtained whose infrared spectrum was identical with that of 3-butyl-3-propyl-1-octanol (II). Its 3,5-dinitrobenzoate was prepared, m.p. 43–44°, and the melting point was not depressed on admixture with authentic 3-butyl-3-propyl-1-octyl 3,5-dinitrobenzoate (IV).

The aqueous solution was evaporated to dryness and the crude potassium salt was converted to its *p*-bromophenacyl ester, m.p. 84–85°; lit.[8] m.p. 85°.

Preparation of pure II by reduction of I. The reduction of 22.6 g. (.094 mole) of I was acccomplished successfully with 3.8 g. (.10 mole) of lithium aluminum hydride followed by destruction of the excess hydride with 100 ml. of 10% sulfuric acid. After work up, there was obtained 18.4 g. (86.4%) of pure II, b.p. 141–142° (2 mm.), n_D^{25} 1.4520.

Preparation of III from II. A mixture of 8.4 g. (.037 mole) of II, 21.6 g. (.212 mole) of acetic anhydride, 20 ml. of dry thiophene-free benzene, and 2 drops of concentrated sulfuric acid was boiled gently for 3 hr. The solution was cooled, poured into 200 ml. of cold, 10% sodium carbonate solution, and allowed to stand for 2 hr. with occasional stirring. The layers were separated, the aqueous layer extracted with benzene, and the combined benzene solutions washed twice with water. The benzene was removed at atmospheric pressure and the residue distilled under reduced pressure. There was obtained 8.7 g. (87.9%) of III, b.p. 140–142° (2 mm.), n_D^{25} 1.4440, whose infrared spectrum was identical with that of the acetate obtained from the separation of the original reaction mixture.

Anal. Calcd. for $C_{17}H_{34}O_2$: C, 75.50; H, 12.67. Found: C, 75.46; H, 12.92.

Treatment of lithium aluminum hydride reduction mixtures with excess ethyl acetate. The reductions listed in Table I were all carried out in the following manner: A solution of .10 mole of the appropriate acid in 100 ml. of dry ether was added dropwise to a slurry of 3.8 g. (.10 mole) of lithium aluminum hydride in 75 ml. of dry ether, and the mixture was refluxed gently for 3 hr. after addition was complete. It was cooled in an ice bath and 50 ml. of a 50% solution of ethyl acetate in ether was added as rapidly as possible, and the mixture allowed to stand for the indicated time. The complex was hydrolyzed by addition of 100 ml. of 10% sulfuric acid and the mixture was worked up and distilled through a short Vigreux column. In the last run a total of 3.3 g. (.0375 mole) of ethyl acetate in 10 ml. of ether was added, this corresponds to an excess of .0125 mole over that required for destruction of the excess hydride. The mixtures obtained after distillation were analyzed by gas chromatography.

Transesterification of ethyl acetate by $(C_8H_{17}O)_4LiAl$. In a 500-ml. three necked flask fitted with a mercury-sealed stirrer, a condenser protected by a drying tube, and a dropping funnel, were placed 0.95 g. (.025 mole) of lithium aluminum hydride and 50 ml. of dry ether, and the mixture was stirred vigorously for 30 minutes. The flask was placed in a constant temperature bath held at 29.1 ± .05°, and 15 g. (.115 mole) of *n*-octyl alcohol in 75 ml. of dry ether added and the mixture was stirred for an additional 2 hr. to insure temperature equilibrium. A solution of 2.2 g. (.025 mole) of ethyl acetate in 10 ml. of dry ether, which had been equilibrated for 2 hr. in the constant temperature bath, was added in one portion. At timed intervals, 5 ml. portions were pipetted from the reaction mixture, quenched with 10 ml. of cold 10% sulfuric acid, and worked up in the usual fashion. Each sample was analyzed by gas chromatography, and the results obtained are given in Table II.

Reduction of 5-nonanone. The reduction of 28.4 g. (.20 mole) of 5-nonanone in 200 ml. of dry ether was carried out using 5.7 g. (.15 mole) of lithium aluminum hydride in 125 ml. of dry ether. The excess hydride was destroyed with 100 ml. of 50% ethyl acetate in ether, the mixture was allowed to stand for 16 hr., and then was hydrolyzed with 150 ml. of 10% sulfuric acid. After work up, there was obtained 23.7 g. (82.3%) of 5-nonanol, b.p. 90–92° (20 mm.), n_D^{25} 1.4360, whose infrared spectrum showed no traces of carbonyl adsorption.

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(8) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed., J. Wiley and Sons, Inc., New York, 1956, p. 276.

Non-lability of the α-Hydrogen during Ninhydrin Oxidation of Alanine[1]

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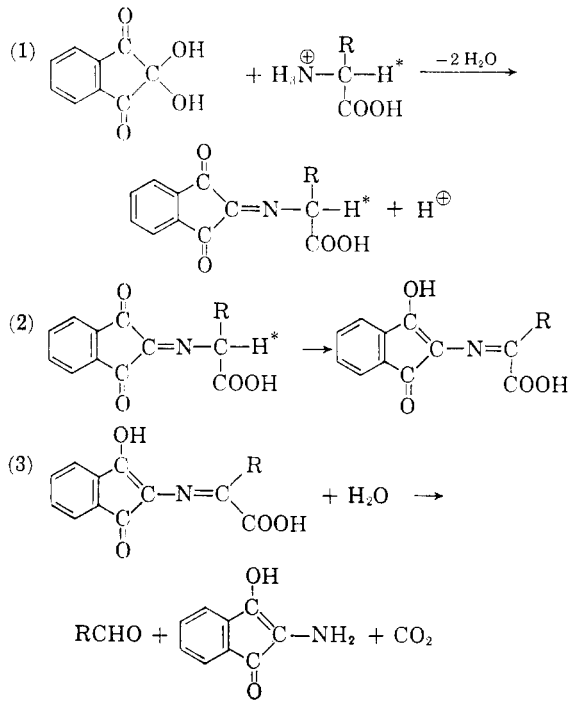
Recent studies of the reactions of recoil tritium with L(+)-alanine required a degradation method which would remove tritium radioactivity from the α-hydrogen position without affecting any radioactivity in the β-positions.[2] An examination of the proposed mechanism for the oxidation of α-amino acids by ninhydrin showed the original condensation reaction (1), followed by enolization (2) and subsequent hydrolysis (3):[3]

(1) Research supported by A.E.C. Contract No. AT-(11-1)-407.

(2) J. G. Kay, R. P. Malsan, and F. S. Rowland, *J. Am. Chem. Soc. in press.*

(3) A. Schönberg and R. Moubasher, *Chem. Revs.*, **50**, 261 (1952). R. Moubasher and M. Ibrahim, *J. Chem. Soc.*, 702 (1949). A. Schönberg, R. Moubasher, and A. Mostafa, *J. Chem. Soc.*, 176 (1948). F. G. Baddar, *J. Chem. Soc.*, *S*, 163 (1949).



As shown above, any tritium activity in the α-position of the amino acid would be exchanged with the solvent and lost during tautomerization; the radioactivity of the resulting acetaldehyde (or derivative) would then serve as a measure solely of the original β-hydrogen radioactivity. However, actual experiments with recoil labeled DL-alanine showed only a minor loss of tritium activity during the reaction sequence from alanine to acetaldehyde derivative, as shown in Table I (a). (Degradation of another aliquot of the same labeled alanine to thallous acetate demonstrated that a large percentage of the tritium activity was originally present in the α-position.)

An additional experiment was then performed in which unlabeled dl-alanine was oxidized in the presence of high activity HTO. The resulting derivative

TABLE I

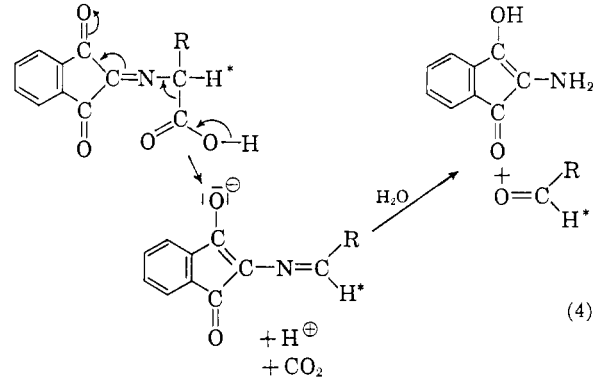
RADIOACTIVITY OF ALANINE AND DERIVATIVES (DPM/μ MOLE)

	(a)	(b)
Alanine	1.32×10^3	0
Water	...	740
Acetaldehyde,-2,4-dinitrophenylhydrazone	1.16×10^3	<0.3
Acetaldehyde dimedone	1.22×10^3	...
Thallous acetate	0.48×10^3	...

of acetaldehyde was essentially non-radioactive [Table I (b)], showing that the α-hydrogen is nonlabile throughout the entire reaction. The small loss of original activity in part (a) of Table I is consistent with previously measured exchange rates for formation of these derivatives.[4]

(4) W. J. Hoff, Jr., and F. S. Rowland, *J. Am. Chem. Soc.*, 79, 4867 (1957).

Under these reaction conditions the mechanism of the ninhydrin oxidation cannot involve tautomeric forms, as in (2), which require hydrogen loss from the α-carbon. The reactions shown in (4) are consistent with this requirement.



EXPERIMENTAL

A 50-mg. sample of the purified tritium-labeled alanine [Table I(a)] was reacted with ninhydrin according to the method of Van Slyke[5] and Wolf[6] using citrate buffer and sweeping the solution with nitrogen while warming on the steam bath. The nitrogen stream swept the acetaldehyde from the reaction into the bottom of an ice-cooled solution of 2,4-dinitrophenylhydrazine dissolved in CH_3OH and HCl. The acetaldehyde-2,4-dinitrophenylhydrazone produced in the alcohol solution was then purified by recrystallization to constant specific activity from methanol-water solutions. This procedure was repeated, substituting a solution of 5,5-dimethylcyclohexane-1,3-dione in 50% aqueous methanol in place of the dinitrophenylhydrazine solution previously used. The dimedone derivative was formed by adding one drop of pyridine and boiling for 30 sec. and was then purified by recrystallization from methanol-water mixtures to constant specific activity.

Another sample (289 mg.) of the purified tritiated alanine was similarly reacted with ninhydrin, sweeping the acetaldehyde product into an ice cooled solution of CrO_3 in M H_2SO_4. After the ninhydrin reaction was complete and all of the acetaldehyde had been swept from the solution, the chromic acid solution was allowed to stand at room temperature overnight in order to complete the oxidation of acetaldehyde to acetic acid. The acetic acid was then removed by steam distillation and titrated with thallous hydroxide according to the method of Wolf and coworkers.[7] The thallous acetate was recrystallized to constant specific activity from methanol-acetone mixtures.

A 68 mg. sample of non-tritium-labeled DL-alanine [Table I(b)] was submitted to the ninhydrin oxidation in the presence of 100 ml. of tritium-labeled water (sp. act = 740 DPM/μ mole), forming acetaldehyde-2,4-dinitrophenylhydrazone from the aldehyde produced and purifying as before.

All specific activities were measured by combustion of a few mg. of the solid according to the method of Wilzbach, Kaplan, and Brown[8] and counting in a silver-walled glass proportional counter with propane as the counter gas.

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(5) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen, and P. Hamilton, *J. Biol. Chem.*, 141, 627 (1941).

(6) A. P. Wolf, private communication.

(7) A. P. Wolf, C. S. Redvanly, and R. C. Anderson, *J. Am. Chem. Soc.*, 79, 3717 (1957).

(8) K. Wilzbach, L. Kaplan, and W. G. Brown, *Science*, 118, 522 (1953).