# Methods for the synthesis of L-lactyl-L-valine-1-$^{14}$C[1]

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The preparation of L-lactyl-L-valine from L-lactic acid and L-valine, by methods used for the synthesis of peptides, was investigated. Initial experiments with *O*-benzoyl-DL-lactic acid and DL-valine benzyl ester unexpectedly gave *N*-benzoyl-DL-valine benzyl ester. However, coupling between *O*-benzyloxycarbonyl-DL-lactic acid and either DL-valine, or DL-valine benzyl ester, occurred in good yield. A simpler method, which was reported to give a 39% yield of L-lactyl-L-valine, gave only 10% of product but was modified to give L-lactyl-L-valine-1-$^{14}$C in 90% yield.

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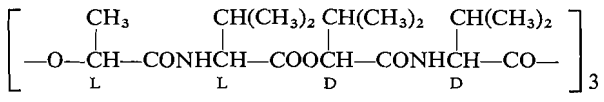

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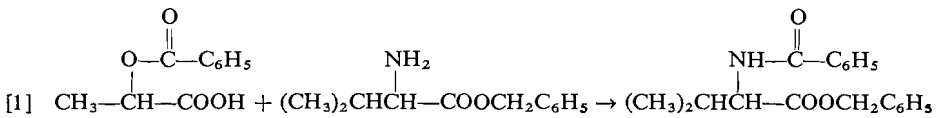

**2**

Previous studies (1) on the biosynthesis of valinomycin (**1**) have shown that L-valine-1-$^{14}$C is distributed equally between the L- and D-valine moieties and that D-valine is not incorporated. In view of these results, the preparation of L-lactyl-L-valine-1-$^{14}$C was undertaken so that this compound could be used for further investigation of the biosynthesis of valinomycin.

As the essential step in the synthesis required the formation of an amide bond between L-lactic acid and L-valine, a number of methods of peptide synthesis, for which good retention of optical purity has been reported (2), were investigated. The intended method envisaged the condensation of *O*-benzyloxycarbonyl-L-lactic acid and L-valine benzyl ester since the protecting groups on these two compounds are easily removed by hydrogenolysis (2). However, because *O*-benzyloxycarbonyl-DL-lactic acid was not readily obtainable in crystalline form the corresponding *O*-benzoyl derivative was used for the initial investigations on racemic compounds.

The condensation reactions were performed using the substituted phosphite ester technique (2), ethoxyacetylene (3), or benzenesulfonyl chloride (4); however, these methods gave (See eq. [1]) a new and unexpected product, which was shown to be *N*-benzoyl-DL-valine benzyl ester (**2**).

The acid chloride method of peptide synthesis (2) was no more successful than the foregoing procedures, and *N*-benzoyl-DL-valine benzyl ester (**2**) was obtained whether phosphorus pentachloride or thionyl chloride was used to generate the acid chloride.

These results were difficult to explain until the reactions were reinvestigated. It was then found that *O*-benzoyl-DL-lactic acid reacted with phosphorus pentachloride and benzenesulfonyl chloride to give benzoyl chloride and benzoic anhydride, respectively. The effects of the substituted phosphite esters and of ethoxyacetylene were not reexamined. However, it seems probable that the result would be production of benzoic anhydride, since ethoxyacetylene reacts with carboxylic acids to give anhydrides (5,6), as does benzenesulfonyl chloride (4), and the phosphite esters produce mixed anhydrides with acyl amino acids (2).

Phosphorus pentachloride did not react with

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*O*-benzyloxycarbonyl-DL-lactic acid to give benzyloxycarbonyl chloride. The nuclear magnetic resonance (n.m.r.) spectrum indicated that the main product was *O*-benzyloxycarbonyl-DL-lactyl chloride together with impurities. When DL-valine benzyl ester was treated with this crude product the result was an oil whose elemental analysis did not indicate *O*-benzyloxycarbonyl-DL-lactyl-DL-valine benzyl ester. However, this compound appears to have been produced, since hydrogenolysis (2) gave DL-lactyl-DL-valine which was characterized as the dicyclohexylamine salt (7).

The required compound was also obtained using a method of peptide synthesis introduced by Birkofer and Ritter (8). In this procedure, *O*-benzyloxycarbonyl-DL-lactic acid *p*-nitrophenyl ester and DL-valine were fused with *N*-trimethylsilylacetamide (9). The product was again an oil which did not give a satisfactory elemental analysis. However, hydrogenolysis (2) gave DL-lactyl-DL-valine identical with the previously obtained compound.

While this work was in progress the synthesis of L-lactyl-L-valine was reported by Shemyakin and co-workers (7). The Russian group first resolved DL-lactic acid and the L-isomer was then converted in turn to the benzyl ester, the hydrazide, and the azide. The azide, in ethyl acetate, was added to an aqueous solution of L-valine and triethylamine. The reported yield of L-lactyl-L-valine was 39% but this could not be duplicated in our laboratory, the best yield being 10% of product which was always contaminated with benzyl alcohol.

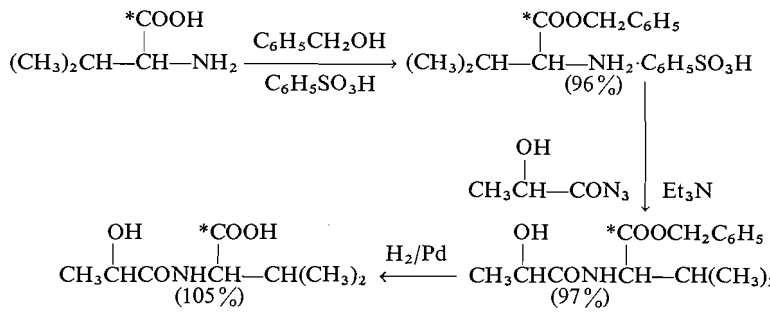
The foregoing method involved a two-phase system and was complicated by the precipitation of L-valine during the reaction. A homogeneous reaction in which L-valine benzyl ester was treated with L-lactic azide, using ethyl acetate as the common solvent, was therefore examined. Using equimolar quantities of L-lactic hydrazide and L-valine benzyl ester good yields (60–70%) of L-lactyl-L-valine benzyl ester were obtained together with unreacted L-valine benzyl ester (40–30%). In order to achieve a high conversion of benzyl-L-valine to L-lactyl-L-valine benzyl ester it was necessary to use a 2.5 mole excess of L-lactic hydrazide. This resulted in a 95% yield of the desired product, or 100% based on benzyl-L-valine used.

The L-lactyl-L-valine benzyl ester thus obtained was always contaminated with solvent and occasionally with a small amount of compound absorbing at 2150 $cm^{-1}$, corresponding to an azide (10). The solvent could be removed by prolonged heating at 50° under vacuum (0.05 mm) and if the other contaminant was present it would sublime out of the flask. The infrared spectrum (KBr) of the sublimate showed an intense band at 2150 $cm^{-1}$. This impurity could be removed by washing an ether solution of the product several times with water or by warming the reaction mixture to 40–45° for 2 h prior to isolating the product. L-Lactyl-L-valine benzyl ester obtained in this way had n.m.r. and infrared spectra consistent with the expected structure.

The hydrogenolysis step proved to be very rapid and gave a quantitative yield of L-lactyl-L-valine. Again, the product was always contaminated with solvent, as indicated by the yield (105%). Complete removal of solvent by warming under vacuum was not feasible due to the tendency of L-lactyl-L-valine to form a lactone (11). The L-lactyl-L-valine, however, could be characterized as the dicyclohexylamine salt (7).

The specific rotations of L-lactyl-L-valine and its dicyclohexylamine salt are given (7) as +8.5° and —13°, respectively. The corresponding products synthesized in this laboratory from commercially available L-lactic acid and L-valine had specific rotations of +9.5° and —8.9°, respectively. There is reasonable agreement on the specific rotation of L-lactyl-L-valine but this is not the case for the dicyclohexylamine salt. In order to resolve this discrepancy, valinomycin was hydrolyzed with dilute methanolic sodium hydroxide (12) to give D-α-hydroxyisovaleryl-D-valine and L-lactyl-L-valine. The specific rotation of D-α-hydroxyisovaleryl-D-valine dicyclohexylamine salt (+25.9°) was in good agreement with the value (+24°) for the product synthesized by Shemyakin *et al.* (7). However, the specific rotation of the L-lactyl-L-valine dicyclohexylamine salt (—8.7°) was close to the value (—8.9°) obtained for the synthetic product from this laboratory.

As an alternative to benzyl L-valine, the use of L-valine *t*-butyl ester was investigated. This had the advantage that the L-lactyl-L-valine *t*-butyl ester was a solid readily purified by crystallization. However, the yield of L-valine *t*-butyl ester




(53%) was considerably lower than that (96%) of L-valine benzyl ester benzenesulfonate.

The best method of preparing L-lactyl-L-valine-1-$^{14}$C appeared to be by formation of the amide bond between L-lactic azide and L-valine-1-$^{14}$C benzyl ester. The desired compound was therefore synthesized according to the following scheme (yield of each step in brackets) in an overall yield of 90% based on the L-valine used and on the L-lactyl-L-valine theoretically obtainable from the L-lactyl-L-valine benzyl ester.

The specific activity of the product of each step is shown in Table I. It is seen that the specific activities of the crystalline products are very similar while those of non-crystalline products are somewhat lower. Using the values in Table I, the purity of L-lactyl-L-valine, compared to the L-valine, is 90%.

TABLE I

Specific activity of products

| Compound | Specific activity (μc/mmole) |
|---|---|
| L-Valine | 2.57 |
| Benzyl-L-valine benzenesulfonate | 2.53 |
| Benzyl L-lactyl-L-valine | 2.39 |
| L-Lactyl-L-valine | 2.31 |
| L-Lactyl-L-valine dicyclohexylammonium salt | 2.50 |

## Experimental

Melting points are uncorrected and were determined on a Fisher–Johns or Thomas–Hoover apparatus (latter for sealed capillaries). Petroleum ether (Skelly F.) had b.p. 30–60°. Infrared spectra were recorded on a Perkin–Elmer 257 grating spectrophotometer, and nuclear magnetic resonance spectra on a Varian H-100 instrument. Optical rotations were measured on a Rudolph polarimeter.

*O-Benzoyl-*DL*-lactic Acid*

A commercially available solution of DL-lactic acid (20 ml, 85%) was extracted with ether overnight and the dried ($MgSO_4$) extract evaporated at 30° under reduced pressure. The residue (5.5 g, 0.061 mole) was dissolved in pyridine (100 ml), cooled in ice, and freshly distilled benzoyl chloride (9.4 g, 0.067 mole) was added slowly with stirring. After standing at room temperature overnight the mixture was poured onto crushed ice, acidified with concentrated HCl and extracted with ether. Evaporation of the washed ($H_2O$) and dried ($MgSO_4$) extract gave a colorless gum (9.6 g) which crystallized from ether – petroleum ether to give *O*-benzoyl-DL-lactic acid (7.5 g, 63%), m.p. 111–113° (lit. (13) m.p. 111.5°).

DL-*Valine Benzyl Ester Benzenesulfonate*

DL-Valine (4.68 g, 0.04 mole), benzenesulfonic acid monohydrate (7.74 g, 0.044 mole), and benzyl alcohol (28 ml, 0.27 mole) in carbon tetrachloride (100 ml) were heated under reflux for 16 h and the condensate dried by passage through silica gel (14). A white crust, which adhered to the wall of the reaction flask, formed rapidly and did not dissolve during the reaction. The mixture was filtered hot and the white solid washed with hot carbon tetrachloride. Dilution of the filtrate with ether and cooling (0°) gave DL-valine benzyl ester benzenesulfonate (12.9 g, 88%), m.p. 152–154°. Recrystallization from ethanol–ether raised the m.p. to 156–157°.

Anal. Calcd. for $C_{18}H_{23}NO_5S$: C, 59.2; H, 6.3; N, 3.8; S, 8.8. Found: C, 59.1; H, 6.6; N, 4.0; S, 9.0.

The white precipitate (0.94 g, 8.5%) had m.p. 167–169°, raised to 170–171° by crystallization from ethanol–ether.

Anal. Calcd. for $C_{11}H_{17}NO_5S$: C, 47.9; H, 6.2; N, 5.1; S, 11.6. Found: C, 47.7; H, 6.4; N, 4.8; S, 11.7.

DL-*Valine Benzenesulfonate*

DL-Valine (117 mg, 1.0 mmole) and benzenesulfonic acid monohydrate (194 mg, 1.1 mmole) were dissolved in water (5 ml) and evaporated to dryness under reduced pressure at 60°. The residue was crystallized from ethanol–ether to give white crystals, m.p. 167–169°. A mixture m.p. with the foregoing product was not depressed and the infrared spectra (KBr) of the two compounds were identical.

DL-*Valine Benzyl Ester*

DL-Valine benzyl ester benzenesulfonate (3.65 g, 0.01 mole) in 5% aqueous potassium bicarbonate (100 ml) was shaken at room temperature for 30 min. Extraction of the aqueous mixture with ether and evaporation of the washed and dried extract gave a pale-yellow oil (1.93 g)

which was distilled to give DL-valine benzyl ester (1.73 g, 84%), b.p. 113–115° at 2mm.

Anal. Calcd. for $C_{12}H_{17}NO_2$: C, 69.5; H, 8.3; N, 6.8. Found: C, 69.5; H, 8.1; N, 6.5.

*Reaction of O-Benzoyl-*DL*-lactic Acid and Benzyl-*DL*-valine with Tetraethylpyrophosphite*

A mixture of benzyl-DL-valine (207 mg, 1.0 mmole), *O*-benzoyl-DL-lactic acid (199 mg, 1.0 mmole), diethylphosphite (680 mg, 5.0 mmole), and tetraethylpyrophosphite (290 mg, 1.1 mmole), was heated on a steam bath for 30 min (15). The mixture was cooled in ice, diluted with cold (0°) water, and extracted with ether. The ether extract was washed with water, 5% potassium bicarbonate solution, and water. Neutralization of the bicarbonate extract with 2 *N* HCl gave *O*-benzoyl-DL-lactic acid (109 mg, 58%), m.p. 108–110° while evaporation of the dried ether extract gave a yellow oil (199 mg) which could not be induced to crystallize. The product in benzene – petroleum ether (1:3) was chromatographed on deactivated (16) Spence type 'H' alumina (6 g) to give *N*-benzoyl-DL-valine benzyl ester (90 mg, 28%), m.p. 73–74° from diisopropyl ether – petroleum ether.

Anal. Calcd. for $C_{19}H_{21}NO_5$: C, 73.3; H, 6.8; N, 4.5. Found: C, 73.2; H, 7.0; N, 4.6.

*Benzoylation of* DL*-Valine Benzyl Ester*

DL-Valine benzyl ester (1 g, 0.0048 mole) in pyridine (10 ml) was cooled in ice and freshly distilled benzoyl chloride (0.63 ml, 0.0053 mole) added. After standing at room temperature overnight the mixture was poured on to crushed ice, acidified with 2 *N* HCl, and extracted with ether. Evaporation of the washed ($H_2O$, 5% $KHCO_3$, $H_2O$) and dried extract gave an oil which solidified on standing. Crystallization from diisopropyl ether – petroleum ether gave *N*-benzoyl-DL-valine benzyl ester (1.21 g, 80%), m.p. 73–74°. A mixture m.p. with the product from the previous reaction was not depressed and the infrared spectra (KBr) of the two products were identical.

*Reaction of O-Benzoyl-*DL*-lactic Acid and Benzyl-*DL*-valine with Diethylchlorophosphite*

A mixture of DL-valine benzyl ester (1.22 g, 0.0059 mole), triethylamine (0.6 g, 0.0059 mole), and diethylchlorophosphite (2) (0.93 g, 0.0059 mole) in dry ether (30 ml) was shaken at room temperature for 17 h, filtered, and evaporated under reduced pressure to give a pale-green oil (17). *O*-Benzoyl-DL-lactic acid (1.15 g, 0.0059 mole) in dry toluene (30 ml) was added and the mixture boiled under reflux for 6 h. After standing overnight (16 h) at room temperature, the mixture was washed with water, 5% potassium bicarbonate solution, and water. Evaporation of the dried toluene solution gave a pale-brown gum (1.45 g), which was chromatographed as before to give *N*-benzoyl-DL-valine benzyl ester, m.p. 73–74° (0.69 g, 37%). Acidification of the bicarbonate extract with 2 *N* HCl gave *O*-benzoyl-DL-lactic acid (0.61 g, 52%).

*Reaction of O-Benzoyl-*DL*-lactic Acid and* DL*-Valine Benzyl Ester with Ethoxyacetylene*

A mixture of DL-valine benzyl ester (2.92 g, 0.014 mole), *O*-benzoyl-DL-lactic acid (2.75 g, 0.014 mole), and ethoxyacetylene (4.1 g, 0.06 mole) in ethyl acetate (70 ml) containing 0.5% water (3), was boiled under reflux for 5 h. After standing at room temperature overnight, the mixture was washed with water, 5% potassium bicarbonate solution, water, 2 *N* HCl, and water. Evaporation of the dried ethyl acetate solution gave a pale-brown gum (5.23 g) which was chromatographed as before to give *N*-benzoyl-DL-valine benzyl ester (3.72 g, 71%). Neutralization of the bicarbonate washings gave *O*-benzoyl-DL-lactic acid (0.57 g, 21%), while neutralization of the acid wash and extraction with ether gave a pale-yellow oil (25 mg) whose infrared spectrum was identical with that of DL-valine benzyl ester.

*Reaction of O-Benzoyl-*DL*-lactic Acid and* DL*-Valine Benzyl Ester with Benzenesulfonyl Chloride*

A solution of benzyl DL-valine (168 mg, 0.8 mmole) in toluene (10 ml) was added over 2 h to a boiling solution of *O*-benzoyl DL-lactic acid (194 mg, 1.0 mmole), triethylamine (130 mg, 1.3 mmole), and benzenesulfonyl chloride (88 mg, 0.5 mmole) in toluene (10 ml). Boiling was continued overnight (17 h) and the mixture evaporated under reduced pressure. The residue was dissolved in ether and the solution washed with water, 4 *N* HCl, water, saturated $NaHCO_3$ solution, and water. Evaporation of the dried ether solution gave a colorless gum (157 mg, 50%) whose infrared spectrum was identical with that of *N*-benzoyl-DL-valine benzyl ester. (When the reaction was repeated without benzyl-DL-valine the product was benzoic anhydride, identified by comparison with an authentic sample).

*Reaction of O-Benzoyl-*DL*-lactic Acid and* DL*-Valine Benzyl Ester Benzenesulfonate by the Acid Chloride Method*

(*a*) *With* $PCl_5$

Phosphorus pentachloride (720 mg, 3.5 mmole) was added over 5 min to a solution of *O*-benzoyl-DL-lactic acid (560 mg, 2.9 mmole) in cold (0°) ether (20 ml). The mixture was stirred at 0° for 1 h and at room temperature for 1 h before pouring the mixture onto crushed ice and extracting with ether. The ether extract was washed ($H_2O$, 5% $NaHCO_3$, $H_2O$), dried, and evaporated to give a colorless oil which was dissolved in dry tetrahydrofuran (10 ml). (In a separate experiment the oil was identified as benzoyl chloride by comparison with an authentic sample).

While the above reaction was proceeding, DL-valine benzyl ester benzenesulfonate (1.06 g, 2.9 mmole) and triethylamine (0.92 ml, 6.5 mmole) in tetrahydrofuran (10 ml) were stirred at room temperature for 2 h. The mixture was cooled to −30° and the product from the previous reaction added over 5 min. After stirring at −30° for 1 h, the mixture was stirred at room temperature for 2 h before removing the solvent under reduced pressure at 50°. The residue was acidified with 1 *N* HCl and extracted with ether, and the extract washed with water, saturated potassium bicarbonate solution, and water. Evaporation of the dried extract gave a pale-yellow oil (0.96 g) which solidified on standing. Crystallization from ether – petroleum ether gave *N*-benzoyl-DL-valine benzyl ester (0.76 g, 85%), m.p. 73–74°.

(*b*) *With* $SOCl_2$

*O*-Benzoyl-DL-lactic acid (560 mg, 2.9 mmole) in thionyl chloride (5 ml) was boiled under reflux for 2 h and allowed to stand overnight (16 h) at room temperature. Excess thionyl chloride was removed at 50° under reduced pressure to give a dark-brown oil. The residue

was dissolved in tetrahydrofuran and added to a mixture of DL-valine benzyl ester benzenesulfonate and triethylamine as in (*a*). The product from the reaction was obtained as a brown gum (0.98 g, 108%) which did not solidify on standing. However, the infrared spectrum was identical with that of the crude product obtained in (*a*).

*O-Benzyloxycarbonyl-*DL*-lactyl-*DL*-valine Benzyl Ester*

*O*-Benzyloxycarbonyl-DL-lactic acid (18) (1.14 g, 0.0051 mole) in tetrahydrofuran (10 ml) at −5° was treated over 5 min with $PCl_5$ (1.32 g, 0.0064 mole). The solution was stirred at −5° for 1 h and at room temperature for a further hour before adding it to the following mixture.

DL-Valine benzyl ester benzenesulfonate (1.86 g, 0.0051 mole) and triethylamine (4 ml, 0.029 mole) in tetrahydrofuran (20 ml) were stirred at room temperature for 2 h and then cooled to −30° before adding the above solution over 15 min. The mixture was stirred at −30° for 1 h and at room temperature for 4 h before removing solvent at 50° under reduced pressure. The residue was acidified with 1 *N* HCl and extracted with ether. The ether extract was washed with water, 5% sodium bicarbonate solution, and water. Evaporation of the dried extract gave a brown gum (2.2 g, 104%) which could not be induced to crystallize.

DL-*Lactyl-*DL*-valine*

The foregoing product (2.2 g) in methanol – acetic acid (249:1 v/v, 50 ml) was hydrogenolyzed over palladium oxide (250 mg) for 7 h. The mixture was filtered and evaporated to dryness to give DL-lactyl-DL-valine as a pale-brown gum (0.89 g, 88%). Part of the product (100 mg) was dissolved in ethyl acetate (5 ml) and treated with freshly distilled dicyclohexylamine until no further precipitation occurred. The DL-lactyl-DL-valine dicyclohexylamine salt was recrystallized from ethanol–ether and had m.p. 162–163°.

Anal. Calcd. for $C_{20}H_{38}N_2O_4$: C, 64.8; H, 10.3. Found: C, 65.1; H, 10.2.

*O-Benzyloxycarbonyl-*DL*-lactyl-*DL*-valine*

To a cold (0°) solution of benzyloxycarbonyl-DL-lactic acid (896 mg, 4.0 mmole) in anhydrous tetrahydrofuran (10 ml) was added ethylchloroformate (432 mg, 4.0 mmole) and triethylamine (404 mg, 4.0 mmole). After standing at 0° for 8 min, *p*-nitrophenol (556 mg, 4.0 mmole) was added and the mixture boiled for 3–4 min and then evaporated under reduced pressure. The residual oil was dissolved in ether, washed with 2 *N* HCl and water and the dried solution evaporated under reduced pressure. The *O*-benzyloxycarbonyl-DL-lactic acid *p*-nitrophenyl ester (1.44 g, 4.6 mmole) so obtained was mixed with DL-valine (490 mg, 4.2 mmole) and trimethylsilyl acetamide (9) (1.36 g, 10 mmole) and fused at 80° (8). After stirring at 80° for 22 h the cooled (25°) mixture was treated with saturated potassium bicarbonate solution and extracted with ether. The aqueous phase was acidified with concentrated sulfuric acid and extracted with ether in the usual manner to give a mixture of carboxylic acids (0.99 g). Chromatography on silicic acid using chloroform – benzene – acetic acid (4:1:1 v/v) gave the major component (892 mg, 67%). Further elution with chloroform – methanol – acetic acid (8:1:1) gave a second fraction (82 mg).

The major component was heated at 90° under vacuum (0.1 mm) to remove acetic acid. The product, which could not be induced to crystallize, was hydrogenolyzed over palladium oxide (157 mg) as for the *O*-benzyloxycarbonyl-DL-lactyl-DL-valine benzyl ester. The DL-lactyl-DL-valine obtained was identical (infrared spectrum) with the previously obtained product and gave an identical dicyclohexylamine salt (258 mg). The yield of DL-lactyl-DL-valine dicyclohexylamine salt, based on the DL-valine used, was 22%.

*Hydrolysis of Valinomycin*

Valinomycin (200 mg) was hydrolyzed in dilute methanolic NaOH according to the procedure of Vining and Taber (12). The crude product (277 mg) was chromatographed on silica gel using Neish's method (19) to give D-α-hydroxyisovaleryl-D-valine (120 mg, 103%) and L-lactyl-L-valine (93 mg, 91%), which were characterized as the dicyclohexylamine salts. D-α-Hydroxyisovaleryl-D-valine dicyclohexylamine salt, m.p. 196–197° (sealed capillary), $[\alpha]_D^{23}$ +25.9° (*c*, 1.45 EtOH) (lit. (7) m.p. 197–198°, $[\alpha]_D^{20}$ +24°). L-Lactyl-L-valine dicyclohexylammonium salt, m.p. 174–176° (sealed capillary), $[\alpha]_D^{23}$ −8.7° (*c*, 2.23 EtOH) (lit. (7) m.p. 174–175°, $[\alpha]_D^{20}$ −13°).

L-*Valine-1-*$^{14}$*C Benzyl Ester Benzenesulfonate*

L-Valine-1-$^{14}$C (approximately 20 μc) was added to L-valine (881 mg), $[\alpha]_D^{25}$ +26.5° (*c*, 2.07 5 *N* HCl) and the whole dissolved in water and evaporated to dryness to give L-valine-1-$^{14}$C (891 mg) specific activity 2.57 μc/mmole. This product (888 mg, 0.0076 mole) together with benzenesulfonic acid monohydrate (1.47 g, 0.0084 mole) and benzyl alcohol (8.1 ml, 0.079 mole) in carbon tetrachloride (22 ml) was boiled under reflux for 26 h and the condensate dried with silica gel (14). After standing at room temperature overnight the product was filtered and washed with ether until the smell of benzyl alcohol could no longer be detected. The L-valine benzyl ester benzenesulfonate (2.67 g, 96%) had m.p. 180–182° and specific activity 2.53 μc/mmole, (lit. (14) m.p. 177–180°).

L-*Lactyl-*L*-valine-1-*$^{14}$*C Benzyl Ester*

Ethyl L-lactate (20) (3.10 g, 0.026 mole) and hydrazine hydrate (5.8 ml, 0.12 mole) in ethanol (30 ml) was boiled for 7 h and then evaporated under reduced pressure on a boiling water bath. The residue was stored over concentrated sulfuric acid in a vacuum desiccator to give L-lactic hydrazide (2.75 g, 101%) as a brown gum.

The hydrazide (0.027 mole) was dissolved in 1 *N* HCl (33 ml), cooled to −5°, and treated with sodium nitrite solution (7.6 ml, 0.028 mole). After 5 min the test for nitrite was negative and more sodium nitrite solution (1 ml) was added. After stirring at −5° for 30 min the mixture was extracted with ethyl acetate and the washed ($H_2O$, 2% $NaHCO_3$, $H_2O$) extract added to the following mixture.

L-Valine-1-$^{14}$C benzyl ester benzenesulfonate (2.67 g, 0.0073 mole) in ethyl acetate (60 ml) containing triethylamine (1.2 ml, 0.0085 mole) was stirred in a cold room at 12° for 3 h and then cooled in ice for 15 min prior to adding triethylamine (1.2 ml) and the solution of L-lactic azide in ethyl acetate. The mixture was stirred at 0° for 7 h, at 12° for 14 h, and at 40–45° for 2 h. The reaction mixture was washed with 1 *N* HCl and water, and the

dried solution evaporated to give L-lactyl-L-valine-1-$^{14}$C benzyl ester as a pale-yellow oil (1.97 g, 97%), $[\alpha]_D^{25}$ −25.1° (*c*, 1.98 EtOH), specific activity 2.39 μc/mmole.

The acid wash on neutralization and extraction with ether gave L-valine-1-$^{14}$C benzyl ester (0.05 g, 2%).

*L-Lactyl-L-valine-1-$^{14}$C*

The benzyl L-lactyl-L-valine-1-$^{14}$C (1.92 g, 0.0069 mole) in methanol – acetic acid (50 ml, 249:1 v/v) was hydrogenolyzed over palladium oxide (347 mg). The mixture was filtered and evaporated under reduced pressure to give L-lactyl-L-valine-1-$^{14}$C (1.37 g, 105%), $[\alpha]^{25}$ +9.1° (*c*, 1.46 EtOH) (lit. (7) $[\alpha]^{20}$ +8.5°), specific activity 2.31 μc/mmole.

A sample of the above product was converted to the dicyclohexylammonium salt, m.p. 175–176° (sealed capillary) (lit. (7) m.p. 174–175°), specific activity 2.50 μc/mmole.

*L-Lactyl-L-valine t-Butyl Ester*

Ethyl L-lactate (3.36 g, 0.0285 mole) and hydrazine hydrate (5.5 ml, 0.113 mole) in ethanol (30 ml) was boiled for 7 h and worked up as before to give L-lactic hydrazide (2.90 g, 98%). The hydrazide (0.028 mole) in 1 *N* HCl (35 ml) was cooled to −5° and treated with sodium nitrite solution (8 ml, 0.029 mole). After 5 min more sodium nitrite (1 ml) was required to give a positive test. The mixture was worked up after 30 min and the azide solution added to L-valine *t*-butyl ester (21) (1.57 g, 0.009 mole) and triethylamine (1.5 ml, 0.011 mole) in ethyl acetate (60 ml) at 0°. The mixture was stirred at 0° for 6 h, 12° for 16 h, and at 40–45° for 2 h. The mixture was worked up in the same way as for the lactylvaline benzyl ester to give L-lactyl-L-valine *t*-butyl ester as a pale-yellow solid (1.77 g, 80%). Recrystallization from ether – petroleum ether gave colorless crystals m.p. 83°, $[\alpha]_D^{25}$ −13.9° (*c*, 2.43 EtOH).

Anal. Calcd. for $C_{12}H_{23}NO_4$: C, 58.8; H, 9.5; N, 5.7. Found: C, 58.9; H, 9.5; N, 5.9.

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