pared in an analogous manner. It, likewise, was condensed with benzaldehyde, producing benzaltetrahydroacetophenone (VII), which, in turn, was condensed with ethyl oxalate to form the keto ester (IX).

Carvone (X) was condensed with two molecules of benzaldehyde to yield an amorphous product whose composition was that of dibenzylidenecarvone (XII).

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

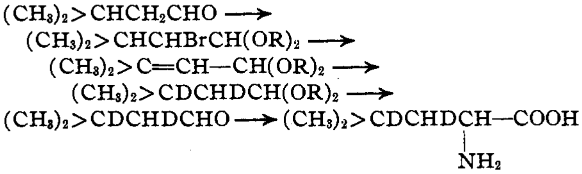
Dideuteriovaline and Dideuterioleucine

BY C. R. KINNEY AND ROGER ADAMS[1]

Analogs of two of the essential amino acids, valine and leucine, have been prepared in which two hydrogen atoms have been substituted by deuterium atoms. These new products are being tested in growth experiments to determine whether they may replace valine and leucine.

It is desirable, if not essential, for the deuterium to be introduced into such positions that during the reactions involved in the synthesis or in the animal body before metabolism actually starts, it will not be replaced by hydrogen. Thus, the deuterium atoms must not be substituted on the amino or carboxyl groups. Moreover, deuterium on a carbon atom from which rearrangement of the deuterium to oxygen or other similar atoms might take place, must be avoided.

The preparation of α-aminoisovaleric-β,γ-d_2 acid (dideuteriovaline) and of α-aminoisohexanoic-β,γ-d_2 acid (dideuterioleucine) from isobutyraldehyde and isovaleraldehyde, respectively, was carried out as illustrated by the following series of reactions for dideuterioleucine



$(CH_3)_2{>}CHCH_2CHO \longrightarrow$

$(CH_3)_2{>}CHCHBrCH(OR)_2 \longrightarrow$

$(CH_3)_2{>}C{=}CH{-}CH(OR)_2 \longrightarrow$

$(CH_3)_2{>}CDCHDCH(OR)_2 \longrightarrow$

$(CH_3)_2{>}CDCHDCHO \longrightarrow (CH_3)_2{>}CDCHD\underset{NH_2}{\underset{|}{CH}}{-}COOH$

It is obvious that in the intermediates isopentanal-β,γ-d_2 and isobutanal-β,γ-d_2, a deuterium on the carbon atom α to the aldehyde group exists; secondary in the former, tertiary in the latter. Both aldehydes theoretically are capable of enolization and thus it is possible that one of the deuterium atoms might be replaced at the intermediate aldehyde stage. A greater tendency to enolization would be expected in the molecule with the tertiary deuterium.

Analyses of the amino acids for deuterium indicated that the dideuteriovaline from the isobutanal-β,γ-d_2 contained about 25% less deuterium and the dideuterioleucine from the isopentanal-β,γ-d_2 6.5% less deuterium than the calculated amount. It is probable that an error of the magnitude of the latter might be introduced in the preparation of the isopentanal-β,γ-d_2 so that any appreciable loss of deuterium through enolization and hydrolysis is not indicated. On the other hand, the error of 25% in the dideuteriovaline probably is due in part to the purity of the isobutanal-β,γ-d_2, but principally to the replacement of deuterium by hydrogen during the transformation of the isobutanal-β,γ-d_2-diethylacetal through the aldehyde to the amino acid. In connection with these operations, it is significant that the deuterium in the intermediates, in spite of rather vigorous conditions in the presence of water and acids, is removed only where enolization may occur and then merely to a minor degree.

The authors desire to express their thanks to Dr. David Rittenberg of the Department of Biochemistry of Columbia University Medical School for his kindness in carrying out the deuterium analyses on the amino acids.

Experimental

α-Bromoisobutyraldehyde Diethylacetal.—Isobutyraldehyde was brominated according to the method[2] used for the preparation of α-bromoisovaleraldehyde diethylacetal. The crude product was fractionated carefully and the product, b. p. 78–82° (28 mm.), used for the subsequent reaction; yield 88%. Completely purified material boils at 80° (28 mm.).

Isobutanal Diethylacetal.—This was prepared according to directions[2] for making isovaleraldehyde diethylacetal except that it was found advantageous after treatment with molten potassium hydroxide to drop water slowly into the hot mixture until sufficient had been added to dissolve

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[1] For the last paper in this field see Leffler and Adams, THIS JOURNAL, **58**, 1555 (1936).

[2] Fischer, Ertel and Loewenberg, *Ber.*, **64B**, 30 (1931); Dworak and Prodinger, *Monatsh.*, **53**, 590 (1929).

all the potassium bromide. The acetal was then separated, dried over solid potassium hydroxide and distilled; b. p. 136–139° (748 mm.); yield 71%.

Anal. Calcd. for $C_6H_{16}O_2$: C, 66.66; H, 11.11. Found: C, 66.52; H, 11.31.

Isobutanal-α,β-d_2 Diethylacetal.—Isobutenal diethylacetal was treated with pure deuterium as described in previous papers[1] using platinum oxide as a catalyst and ethyl acetate as a solvent. A large cell was employed so that the deuterium could be generated rapidly. The pure material which gave no permanganate reaction had a boiling point of 133–135° (747 mm.); $d^{20}{}_4$ 0.8368; $n^{20}{}_D$ 1.3938.

Anal. Calcd. for $C_8H_{16}D_2O_2$: C, 64.86; H + D, 12.31. Found: C, 64.60; H + D, 11.90.

Isobutanal Diethylacetal.—This was prepared by the reduction of the unsaturated acetal with hydrogen. It had the following constants: b. p. 135–136° (745 mm.); $d^{20}{}_4$ 0.8295; $n^{20}{}_D$ 1.3885. Oeconomides[3] reported the b. p., 134–136°; $d^{12}{}_4$ 0.9957.

The calculated value for the density of isobutanal-α,β-d_2 diethylacetal on the assumption of the same molecular volume for deuterium and hydrogen[4] is 0.8408 as compared with the found value of 0.8368.

Dideuteriovaline (α-Aminoisovaleric-β,γ-d_2 Acid).—A mixture of 35 g. of isobutanal-α,β-d_2 diethylacetal (which did not react with potassium permanganate solution in three to four minutes), 125 cc. of water and 15 drops of concentrated sulfuric acid was stirred or shaken for thirty minutes. The reaction product was distilled slowly into a flask cooled in ice, containing 100 cc. of methanol and 35 g. each of ammonium chloride and potassium cyanide. When all of the aldehyde had distilled, the receiver was connected to a reflux condenser and refluxed for two hours. After cooling, 100 cc. of ether was added and the precipitated salt filtered and washed with ether. To the alcohol–ether solution 100 cc. of 40% aqueous hydrogen bromide and 25 cc. of water was added, and the mixture allowed to stand for two hours. It was heated under an air reflux until the alcohol and ether were removed. Some water was added to replace that lost by evaporation. The air reflux was replaced by a water-cooled one and the mixture refluxed for a total of twelve hours.

The solution was carefully evaporated to dryness, the final stages being carried out under reduced pressure. The solid was dissolved in about 200 cc. of water, the solution cooled in ice and well-washed silver oxide was added (from 50 g. of silver nitrate) in small lots. When an excess of silver oxide had been added, the solution was filtered and the precipitate well washed. Hydrogen sulfide was passed into the solution until no more silver sulfide precipitated. After filtering, the solution was evaporated until the amino acid began to crystallize out. Methyl alcohol was added and the solution cooled in ice; yield 12.2 g. (43.4%). The acid was recrystallized from water, using norite to remove a faint yellow color. If heated slowly, the melting point in a sealed tube was 273° with decomposition. The substance consistently melted 1.5–2° lower than pure *dl*-valine when both were melted in the same bath.

Anal. Calcd. for $C_5H_9D_2O_2N$: N, 11.76; D, 18.8 atom per cent. Found: N, 11.54, 11.44; D, 13.2 atom per cent. (±0.7).

Isopentanal-α,β-d_2 Diethylacetal.—Isopentenal diethylacetal was prepared[2] from isovaleraldehyde made from synthetic isoamyl alcohol. Difficulty was encountered in its reduction with deuterium. The best method was found to be by the use of dioxane (distilled from sodium) as a solvent, freshly distilled isopentenal acetal and Raney nickel as a catalyst. The product after fractionation gave a b. p., 164–165° (740 mm.); $n^{20}{}_D$ 1.4025; $d^{20}{}_4$ 0.8423.

Anal. Calcd. for $C_9H_{18}D_2O_2$: C, 66.66; H + D, 13.57. Found: C, 66.21; H + D, 12.59.

Isopentanal Diethylacetal.—This was prepared by reduction of the unsaturated acetal with hydrogen. It had the following constants: b. p. 167–168° (750 mm.); $n^{20}{}_D$ 1.4040; $d^{20}{}_4$ 0.8356. Alsberg[5] reported b. p. 168.2°; $d^{12}{}_4$ 0.835.

The calculated value for the density of isopentanal-α,β-d_2 diethylacetal on the assumption of the same molecular volume for deuterium and hydrogen is 0.8464 as compared with the found value of 0.8423.

Dideuterioleucine (α-Aminoisohexanoic-β,γ-d_2 Acid).—The isopentanal-α,β-d_2 diethylacetal (which did not react with potassium permanganate solution in three to four minutes) was converted to dideuterioleucine following the procedure outlined for the valine derivative. The yield of acid recrystallized from water, using norite to remove color, was about 30% of the weight of the acetal. The melting point taken slowly in a sealed tube was 271° with decomposition. *dl*-Leucine in the same bath melted consistently 2° higher.

Anal. Calcd. for $C_6H_{11}D_2O_2N$: N, 10.52; D, 15.4 atom per cent. Found: N, 10.31; D, 13.7 atom per cent. (±0.7).

Summary

Dideuteriovaline(α-aminoisovaleric-β,γ-d_2 acid) and dideuterioleucine (α-aminoisohexanoic-β,γ-d_2 acid) have been prepared for comparison with valine and leucine in growth experiments.

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(3) Oeconomides, *Bull. soc. chim.*, **35**, 500 (1881).

(4) McLean and Adams, THIS JOURNAL, **58**, 804 (1936).

(5) Alsberg, *Jahresber. Fortschritte Chem.*, 485 (1864).