THE INVESTIGATION OF SOLID-STATE CATALYTIC HYDROGENATION OF ORGANIC COMPOUNDS. II. THE SYNTHESIS OF TRITIUM-LABELLED VALINE

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SUMMARY.

We report here the investigation of solid-state catalytic hydrogenation (SCH) of unsaturated compounds, used for the synthesis of tritium labelled valine The effect of the solid-state components ratio , tritium pressure and reaction temperature on the reaction rate has been investigated. It has been found that the SCH reaction rate depends linearly on tritium pressure in the 5-50 kPa interval. ^{3}H NMR spectroscopy was used to determine the distribution of tritium label in synthesized valine. It was found, that the distribution of tritium label in the product molecules depends both on the precursor compound structure and the reaction temperature. SCH activation energy was determined to be equal to 11 kcal/mol. The application of solid-state isotope exchange for tritium labelling of unsaturated compounds has been shown for 3,3-dimethyl-2-benzoylaminoacrylic acid.

Key Words: Solid-State reaction, Catalytic hydrogenation, Valine.

INTRODUCTION.

Catalytic hydrogenation of unsaturated compounds is widely used for the preparative synthesis of tritium labelled

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compounds. The reaction is usually conducted in organic solvents in the presence of platinum catalysts [1]. Tritium hydrogenation of unsaturated compounds in the solid phase at elevated temperature is a new method which opens new broad avenue for tritium labelling of different compounds of interest [2,3,4]. Hydrogen isotopes are activated on the catalyst, and the hydrogenation reaction proceeds in the organic compound layer. It was suggested that the spillover of activated hydrogen into the organic compound layer takes place during SCH of aromatic phenols [5]. Hydrogen isotopes are activated on the catalyst, and the hydrogenation reaction proceeds in the organic compound layer. However, the nature of activated hydrogen and the mechanism of solid state hydrogenation still remains unknown. The catalytic solid-state hydrogenation during the synthesis of [2,3-^{3}H]alanine from 2-hydroxy-iminopropionic acid was studied in the earlier communication [6]. Using ^{3}H NMR it was shown that the distribution of tritium label in alanine is dependent upon the reaction temperature. In this work we show results of the effect of the ratio between the solid state components,the reaction temperature,tritium pressure and the nature of the initial unsaturated compound on the SCH reaction rate, as well as on the specific activities and label distribution in the products.

EXPERIMENTAL.

1.Synthesis of 2-phenyl-4-isopropylidene-oxazolone-5 (I).

Mixture of 294 ml (4 mol) dry and distilled acetone, 14.3 g (0.08 mol) of hippuric acid, 4.88 g (0.06 mol) of anhydrous sodium acetate was heated to boiling and 20.4 g (18.9 ml, 0.2 mol) of distilled acetic anhydride was added in drops during 30 min. and the boiling mixture was then stirred for 4 h. The excess of acetone was driven off , and the remainder poured on 200 g of ice and distilled water was added to 1000 ml. Oxazolon

(I) precipitated as yellow needles, the product was separated and washed with water and with 4% Na_2CO_3. Product was dried over KOH. The yield was 10 g (62 %), the melting temperature was 97-99 °C. Oxazolone (I) was purified by crystallization from acetone:water (2:1).

2. Synthesis of 3,3-dimethyl-2-benzoylamineacrylic acid (II).

5.2 g of purified oxazalone (I) was added to 4 % alkaline solution, the mixture was heated to boiling with mechanical stirring. After boiling for 4.5-5 h the greenish-yellow solution was cooled, filtered and acidified with 10-12 % HCl to pH 1. Colourless sediment was separated , washed with water, dried over KOH. The weight was 4.1 g (72 %), melting temperature 211-212 °C. Crystallization from aqueous alcohol (1:1) was performed. The resulting product had the melting temperature 217-218 °C. (obtained %: C 65.35 ; H 6.01 ; N 6.02, calculated %: C 65.74 ; H 5.97 ; N 6.39).

3. Synthesis of N-benzoylvaline (III).

1.17 g of valine (0.010 Mol) from "Reanal" is dissolved in 10 ml of 4 M alkali and placed in a reactor equipped with a stirrer and a dropping funnel. The mixture was cooled with icy water and 1.5 g of benzoylchloride (1.3 ml 0.011 mol) was added with mixing ; pH of the reaction was kept above 10 all the time. After stirring for 30 min at room temperature, the mixture was cooled and acidified with drops of concentrated hydrochloric acid. Mixture was left for 2 hrs at 40°C, then the sediment was separated, washed with icy water and dried in air. Benzoic acid was removed from the product by boiling it in 3-4 ml of carbon tetrachloride (CCl_4) and subsequent filtering of the slightly cooled mixture. The yield was 1.8 g (82%), melting temperature 132-132°C; crystallization from hydrous ethanol was performed

(obtained % : C 65.40 ; H 6.40 ; N 6.40 , calculated % : C 65 43; H 6.41 ; N 6.36).

4. Synthesis of [^{3}H]benzoylvaline (IV) and [^{3}H]3,3-dimethyl-2-benzoylaminoacrylic acid (V).

2 mg of acid (II) applied on 20 mg of 5% palladium-on-barium sulphate catalyst was placed in a 10 ml glass vessel. The reaction mixture was evacuated to a pressure of 10^{-1} Pa, and tritium was injected into the vessel to the final pressure 25 kPa. The vessel is heated to 100°C and kept at this temperature for an hour, then cooled and tritium was removed. Tritium-labelled products were washed off with two 1.5 ml portions of acetone. The filtrate was twice evaporated with 5 ml of methanol. The products were dissolved in 1 ml of methanol applied on a 250 mm x 22 mm chromatography column packed with the Nucleosil C_{18} sorbent. Labelled compounds were eluted at 5 ml/min with 35 methanol containing 0.1% trifluoroacetic acid. The retention volumes of (IV) and acid (V) were respectively 240 and 110 ml . Product-containing fractions were collected, evaporated to dryness and dissolved in ethanol to a radioactive concentration of 1 Ci/litre. 18 mCi [^{3}H]3,3-dimethyl-2-benzoylamineacrylic acid with a specific radioactivity of 8.3 Ci/mmol and 230 mCi of [^{3}H]-N-benzoylvaline with a specific radioactivity of 74 Ci/mmol were obtained.

5. Synthesis of [2,3-^{3}H]-valine (VI).

1 mg of oxazolone (VI) was applied on 10 mg of 5% palladium-on-barium sulfate catalyst and treated as described in the previous section. Tritium-labelled products were washed off with two 1.5 ml portion of acetone. Filtrate was evaporated to dryness under reduced pressure. Evaporation was repeated from 5 ml of methanol. The dry matter was boiled in 5 ml of 6 N HCl for an hour and the acid was evaporated to dryness under reduced pressure.

Chromatographic purification was carried out on the Amberlite CG 50 (III) cationic exchanger filled with copper (II) ions. The sorbent was 70% saturated with copper ions The sorbent was placed in a 140 mm x 8 mm column. 0.2 M of ammonium hydroxide was used as eluent. Traces of copper ions were removed from the eluent by passing through a 15 mm x 8 mm chromatographic column packed with Dowex A-1 complex-forming sorbent. Valine-containing fraction (monitored with UV-detector) were collected from 22 to 27 ml. Valine was isolated by chromatography on the Aminex Q-150 S cationic exchanger packed in a 50 mm x 8 mm column. The column was washed with water, valine was desorbed with 1.0 M aqueous ammonia. Eluent was evaporated to dryness under reduced pressure, valine was dissolved in 50% aqueous ethanol to a radioactive concentration of 1 Ci/l.

The analysis of chemical and radiochemical purity was conducted by thin-layer chromatography on "Silufol" silica gel in the isopropanol:acetone:ammonia system (15:9:9). Valine mobility was 0.45, radiochemical purity 98%. 240 mg of [2,3-^{3}H]-valine with specific radioactivity of 50 Ci/mmol was obtained. The chemical yield was 41%. At 120°C [2,3-^{3}H]valine with chemical yield of 46% and specific radioactivity 60 Ci/mmol was synthesized.

6. ^{3}H NMR and ^{1}H NMR spectroscopy.

^{3}H NMR and ^{1}H NMR spectra of amino acid solution were obtained on an AC 250 Bruker spectrometer equipped with a ^{1}H/^{3}H 5 mm dual probe, the operation frequency being 266.8 and 250 MHz, respectively. Chemical shifts of tritium signals in completely tritium-substituted compounds were assumed to be equal to the corresponding shifts of protons. To obtain the spectrum, 50-100 mCi of labelled valine dissolved in 0.5 ml of ^2H_2O were placed in a vial. Spectra of (IV) and acid (V) were obtained in [$^2H_4$]-methanol.

7. The determination of the convertion degree of 3,3-dimethyl-2-benzoylaminoacrylic acid.

0.5 mg of acid (II) applied on 5% palladium-on-barium sulfate catalyst was placed in a 10 ml glass vial. Treatment was conducted as described for the preparative synthesis of (IV). The sample is dissolved in 1 ml of 50% methanol. 20 mkl of solution was applied on a 250 mm x 4mm 5 mkm C8 Zorbax column. Eluent was 30% aqueous methanol containing 0.1% of trifluoroacetic acid. The peaks were monitored with UV-detector at 206 nm. The degree of conversion was calculated from the mass relationship of (IV) and (V).

RESULTS AND DISCUSSION

Fig.1 shows the kinetics of solid state catalytic

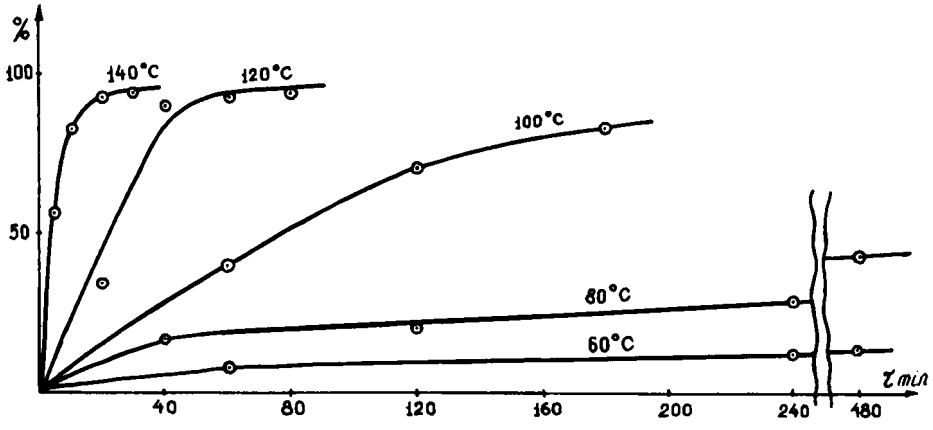

Fig.1 Kinetics of SCH of 3,3-dimethyl-2-benzoylaminoacrylic acid at tritium pressure 25 kPa and the catalyst-substrate ratio 20:1.

hydrogenation. At temperatures of 60°C and less, the reaction is completed long before conversion of the initial compound to the final product occurs. At these temperatures, spillover tritium reacts with the smallest part of the organic compound only. A considerable part of the organic phase layer appears to be inaccessible for this interaction. Reaction stoppage at a low

temperature is not due to catalyst poisoning, since further temperature elevation for 50°-60°C results in the quantitative conversion of the initial product. At low temperatures, spillover tritium rapidly exchanges with hydrogen in labile positions of the organic compound molecule in the whole volume. Thus in as little as 20 min at 20°C the labile tritium is found in the organic compound layer, the labile positions corresponding to two hydrogen atoms in 3,3-dimethyl-2-benzoylaminoacrylic acid. At 60°C spillover tritium diffuses through the whole volume of the organic phase, but its energy is not sufficient for the reaction in the whole volume.

The SCH activation energy of 3,3-dimethyl-2-benzoylaminoacrylic acid, as determined from the linear parts of hydrogenation curves obtained at temperatures of 60-140°C, was 11 kcal/mol. This value is close to that obtained from the high-temperature solid-state catalytic isotope exchange (HSCIE) of valine with gaseous tritium (12 kcal/mol) [9]. The equality of activation energy values for HSCIE and SCH could indicate that both reactions are limited by the same stage, namely the hydrogen spillover, since the well-known value of spillover activation energy on platinum metals is in good agreement with values obtained for solid state reactions of isotope exchange and hydrogenation [10].

Fig. 2 shows the hydrogenation kinetics obtained at hydrogen pressure of 5-25 kPa. The curves are of similar character, but if the reaction is completed in an hour at 25 kPa, less than half of unsaturated compound reacts at 5 kPa by this time. Fig.3 shows the conversion obtained after 20 min of SCH.

Practically linear dependency of the reaction rate on the hydrogen pressure was obtained for the reaction under study. The ten-fold pressure elevation leads to a 5.5-fold increase in the

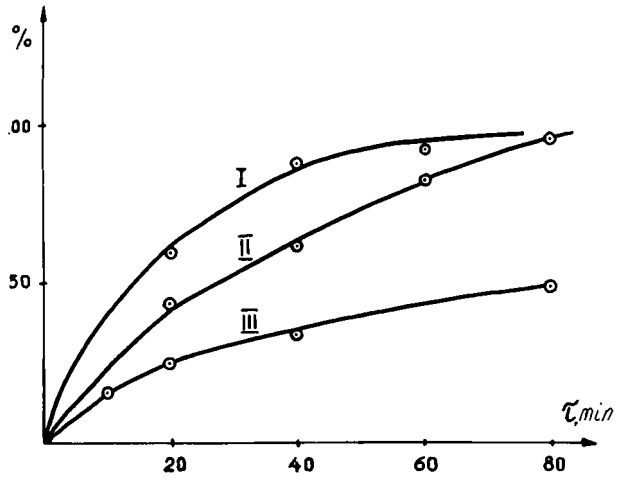

Fig.2 Kinetics of SCH of 3,3-dimethyl-2-benzoylaminoacrylic acid at tritium pressure 25 kPa (I), 15 kPa (II), and 5 kPa (III) at the catalyst-substrate ratio 10:1.

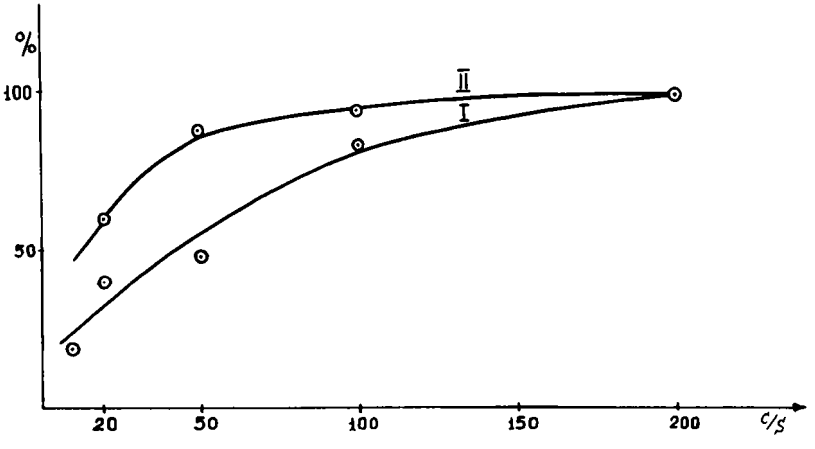

Fig.3 The degree of conversion of 3,3-dimethyl-2-benzoylaminoacrylic acid into benzoylvaline as a function of tritium pressure at 100°C in 20 min.

hydrogenation rate. The observed dependence of the reaction rate on pressure disagrees with the kinetics of radioactive label introduction in thymine on the palladium membrane, which was reported to be pressure-independent [11]. Additional experiments are needed to elucidate this controversy.

The hydrogenation rate can be increased by reducing the

thickness of organic compound layer (Fig.4). At 1:10 compound:catalyst ratio, the yield is 20% only, while the reaction proceeds almost completely at 1:200 ratio under similar condition. Thus to reach a greater conversion of the initial compound in the SCH reaction, hydrogen pressure should be elevated and the catalyst:compound ratio increased.

The temperature elevation of SCH leads not only to the increase of the reaction rate but also changes the tritium

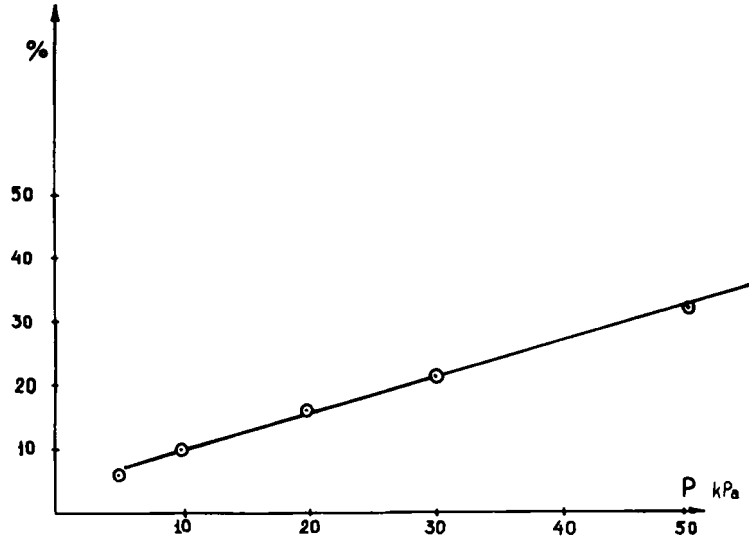

Fig.4 The degree of conversion of 3,3-dimethyl-2-benzoylaminoacrylic acid into benzoylvaline in 20 min (I) and 40 min (II) as a function of the catalyst-substrate ratio (C/S).

distribution in the molecule (Table 2). The temperature increase from 100° to 120°C during SCH of oxazolone (I) results in the increase of specific radioactivity of resulting valine from 50 to 60 Ci/mmol. This increase is mainly due to increased tritium incorporation into the methyl groups. Increasing temperature from 100° to 120°C leads to a 2-fold increase of tritium-substituted hydrogen in the methyl groups. The use of another precursor compound, 3,3-dimethyl-2-benzoylaminoacrylic acid, leads to dramatic changes in the tritium label distribution

Table 1

^3H NMR of tritium labelled valine synthesized by SCH at a temperature of : I-100°C; II-120°C.

Chemical shift (ppm)	Position	Label distribution %		Hydrogen substitution %	
		I	II	I	II
3,60	2	28	25	48	52
2,30	3	50	41	85	86
1,10	4	22	33	6	12

(Table 2). Although the multiple bond in the precursor is situated between the second and third carbon atoms, tritium is incorporated in the allyl position, preferably in the methyl groups. Thus, by modifying the precursor structure, one can achieve considerable changes in the distribution of isotope atoms.

Table 2

^3H NMR of tritium labelled N-benzoylvaline synthesized by SCH at a temperature of 100°C.

Chemical shift (ppm)	Position	Label distribution %	Hydrogen substitution %
7,85	Ph(o)	0	0
7,50	Ph(m,n)	8	6,6
4,50	2	8	20
2,30	3	18	46
1,05	4	66	28

Tritium-labelled acid (V) was obtained by the solid state isotope exchange reaction (Table 3). Tritium label was exclusively incorporated in the methyl groupings, the 4 -th methyl group being labelled two times as much as the 4-th one. The 4´-th group is located in a stronger field on the ^1H NMR spectrum. Tritium label was not incorporated in the benzoyl

Table 3

^3H NMR of tritium labelled 3,3-dimethyl-2-benzoylaminoacrylic acid synthesized by solid state isotope exchange at 100°C

Chemical shift (ppm)	Position	Label distribution %
2,25	4	35
1,9	4	65

group of 3,3-dimethyl-2-benzoylaminoacrylic acid under conditions used. This means that solid state reactions can be used for label incorporation in unsaturated compounds without multiple bond hydrogenation.

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