Syntheses of tritium-labeled surface-active substances*

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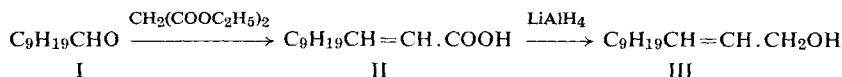
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SUMMARY

Dodecanoic-2,3-^3H, ^3H acid was synthesized by addition of tritiated hydrogen to 2-dodecenoic acid which was obtained by bromination and subsequent dehydrobromination of dodecanoic acid. Urushibara nickel has proven to be effective for the hydrogenation. Reduction of dodecanoic-2,3-^3H, ^3H acid with lithium aluminium hydride led with good yield to 1-dodecanol-2,3-^3H, ^3H from which sodium dodecyl-2,3-^3H, ^3H sulfate was synthesized successfully. Advantage of the present method includes the easiness of separation of resultant from reactant mixture at each stage of the synthetic route. The method seems to assure the obtaining of the surface-active substances whose purities are high enough to be used for determining the adsorbed amount by means of radiotracer method.
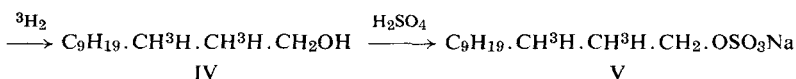
INTRODUCTION

Owing to the extremely soft nature of its β-rays, tritium has been expectea to be the most effective radioisotope for measuring the adsorbed amounts of surface-active substances at their solution surfaces. Few papers [1, 2], however, have been published in this field, because of the difficulties to determine the radioactivity over the solution surface and to synthesize the tritiated surface-active substances, whose purity satisfies radiochemical and surface-chemical requirements. Studies have thus been carried out in our laboratory to overcome the former difficulty by developing the counting methods [3,4] useful for solution surfaces. The present paper deals with the syntheses of several amphipathic compounds which are pure enough to be used for the adsorption experiments.

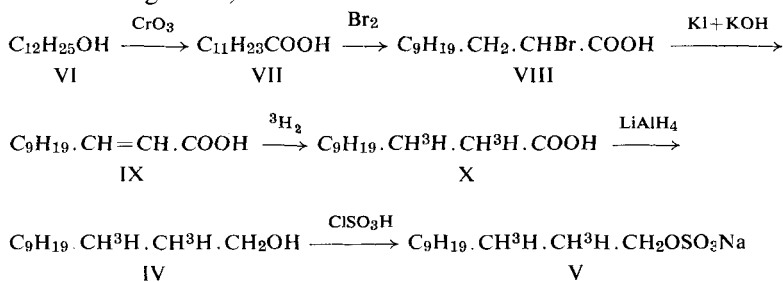Nilsson [1] has reported the following synthetic route leading to the tritiated sodium dodecyl sulfate (V), (SDS);

$$C_9H_{19}CHO \xrightarrow{CH_2(COOC_2H_5)_2} C_9H_{19}CH=CH.COOH \xrightarrow{LiAlH_4} C_9H_{19}CH=CH.CH_2OH$$

$$\text{I} \qquad\qquad\qquad \text{II} \qquad\qquad\qquad \text{III}$$

$$\xrightarrow{^3H_2} C_9H_{19}.CH^3H.CH^3H.CH_2OH \xrightarrow{H_2SO_4} C_9H_{19}.CH^3H.CH^3H.CH_2.OSO_3Na$$

$$\qquad\qquad IV \qquad\qquad\qquad\qquad\qquad\qquad V$$

Since then, however, no successful results have been reported with the above route. It has been pointed out by many investigators [5] that the condensation reaction, I → II, involves some uncertainty. According to our experiences [6], the condensation product of I and diethyl malonate, even after the repeated purification procedures, absorbed hydrogen gas no more than 50% to the theoretical value, the fact suggesting the incompletion of the condensation. Moreover, resultant product at any stage of the route was extremely difficult to separate from the reactant mixture and by-products. Even though we performed repeated purifications at each stage for products II to IV, the surface tension-concentration curve for the final product (V) showed by any means a depression which could be an indication of impurities due probably to the inadequate synthetic processes.

Similar results were obtained by Nilsson himself [1] who erased the depression by isotopic dilution of the impure radioactive product with pure non-radioactive SDS. However, such an isotopic dilution results evidently in the lowering of radioactive purity; if accompanying impurities are radioactive, their specific activity is relatively increased by the dilution. It is necessary procedure in radioactive syntheses of surface-active substances to confirm non-existence of impurities or to make sure that the impurities, if any, are little in their contents, evidently negligible in their specific activity, and very low in their surface activity, in comparison with the main component. To attain this purpose, isotopic dilution should be made for lowering specific activity not of the main component, but of the impurities which should be removed subsequently. What Nilsson has done is contrary to this principle.

To obtain tritiated dodecanol and SDS of extremely high purity, we took the following route;

$$C_{12}H_{25}OH \xrightarrow{CrO_3} C_{11}H_{23}COOH \xrightarrow{Br_2} C_9H_{19}.CH_2.CHBr.COOH \xrightarrow{KI+KOH}$$

$$\quad VI \qquad\qquad\quad VII \qquad\qquad\qquad VIII$$

$$C_9H_{19}.CH=CH.COOH \xrightarrow{^3H_2} C_9H_{19}.CH^3H.CH^3H.COOH \xrightarrow{LiAlH_4}$$

$$\quad IX \qquad\qquad\qquad\qquad\qquad X$$

$$C_9H_{19}.CH^3H.CH^3H.CH_2OH \xrightarrow{ClSO_3H} C_9H_{19}.CH^3H.CH^3H.CH_2OSO_3Na$$

$$\quad IV \qquad\qquad\qquad\qquad\qquad\qquad V$$

Convenience of this route includes the easiness of separation of a resultant from the reactant system at each stage. Each product was purified repeatedly by fractional distillation and, if necessary, fractional crystallization at the expense of yield, until it would meet the purity criteria. The identification procedures comprised elemental analyses, chemical modifications to derivatives, hydrogen

absorption (for IX), visible and infrared absorption, paper- and/or gaschromato-graphies. Dodecenoic acid (IX), for example, was identified by elemental analyses and melting point determinations for the acid, its amide, hydrogenated product (same as VII) and its amide. In addition, phenylisocyanate test proved that the LiAlH$_4$-reduced product from the hydrogenated IX was identical to the original 1-dodecanol (VI).

The synthetic route, VII → IX, was nearly the same as the Meyers' method which had proven to be effective in synthesizing 2-octadecenoic acid [7]. Tritiation of IX was done with the apparatus shown in figure 1. The amount of tritium gas (by AERE, Harwell, England) in the ampoule A in the figure was determined
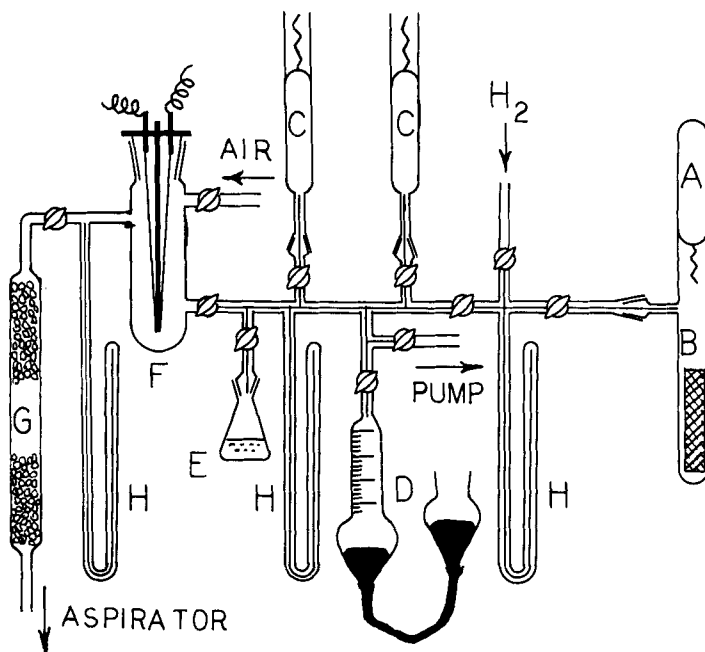

FIG. 1. — Tritiation apparatus.

A	Tritium ampoule.	E	Reaction flask.
B	Breaker.	F	Combustion bulb.
C	Ampoule for storage.	G	CaCl$_2$ tube.
D	Graduated Toepler.	H	Manometer.

by measuring the pressure elevation of the vacuum line upon breaking the ampoule. Isotopic abundance, $^3H/(H+^3H)$, of tritium was found mass-spectrometrically to be 96%. Thus, 240 mC portion of tritium was introduced into the flask E containing IX, solvent, and Urushibara nickel (U-Ni), which has proven to make it very easy to hydrogenate various alkene compounds with its catalytic activity comparable with that of Raney nickel [8]. Figure 2 gives the absorption

curve which shows an agreement of the saturation value of hydrogen gas (labeled with tritium) with theoretical. After the tritiation, product was treated with methanol for isotopic dilution of labile tritium atoms which might have been incorporated into the carboxyl and other positions in the molecule of dodecanoic-2,3-^{3}H, ^{3}H acid. The procedure comprising the dissolution and subsequent evaporation of methanol was repeated to decrease specific activity of the labile tritium to as low as 2×10^{-16} times the value prior to exchange. Radioactive yield was found to be 71.2%, while ca. 72% was found to be the chemical yield for the purified acid.
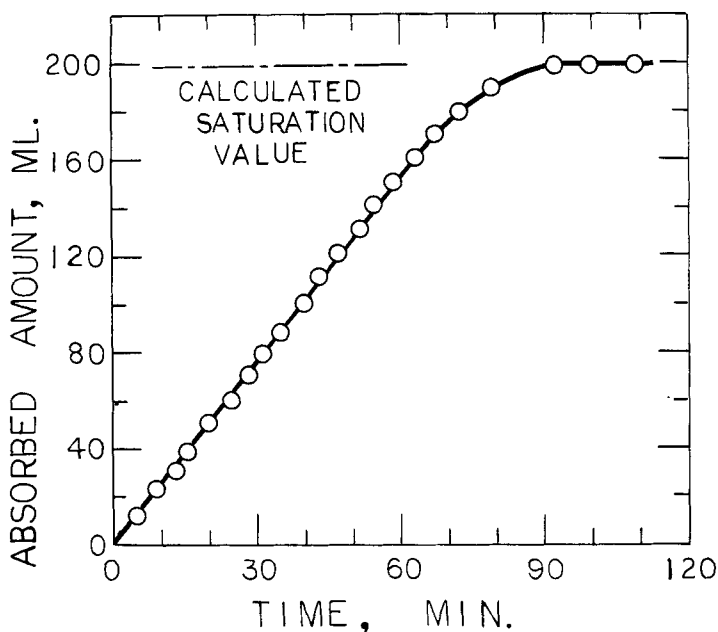

FIG. 2. — Absorption of gaseous hydrogen labelled with tritium by 2-dodecenoic acid.

2-dodecenoic acid, 1.66 g.   Catalyser, 0.5 g of U-Ni.
Tritium, 240 mC.   Solvent, dioxane 10 ml.

Figure 3 illustrates the paper chromatogram for various fatty acids which have been treated with p-bromophenacyl bromide and 2,4-dinitrophenyl-hydrazine [9]. It can be seen in the figure that all of the upper spots, X, are on nearly the same position, regardless of the chain length of acids, while the lower ones, Y, take the positions proper for each chain length. It must be mentioned that the mere mixture of p-bromophenacyl bromide and 2,4-dinitrophenyl-hydrazine has given the upper spot only. It is thus concluded that the tritiated X is chemically identical to the pure sample of non-radioactive VII. In figure 4, the solid line expresses the 420 mμ absorbance over the chromatogram for the product X, while the dotted one gives the distribution of tritium radioactivity
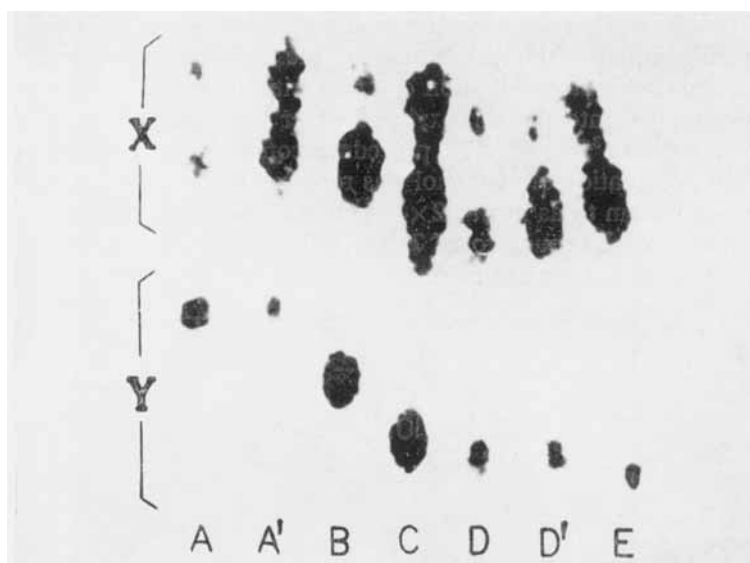
FIG. 3. — Paper chromatogram for 2,4-dinitrophenylhydrazones of p-bromophenacyl esters of (A) dodecanoic acid, (A′) dodecanoic-2,3-^{3}H, ^{3}H acid, (B) tetradecanoic acid, (C) hexadecanoic acid, (D) octadecanoic acid, (D′) octadecanoic-9,10-^{3}H, ^{3}H acid, and (E) eicosanoic acid. *Solvent system :*
Stationary phase, petroleum hydrocarbon (b.p. 150-180 °C).
Moving phase, methanol-acetic acid-petroleum hydrocarbon (20:2:1 in volume ratio).
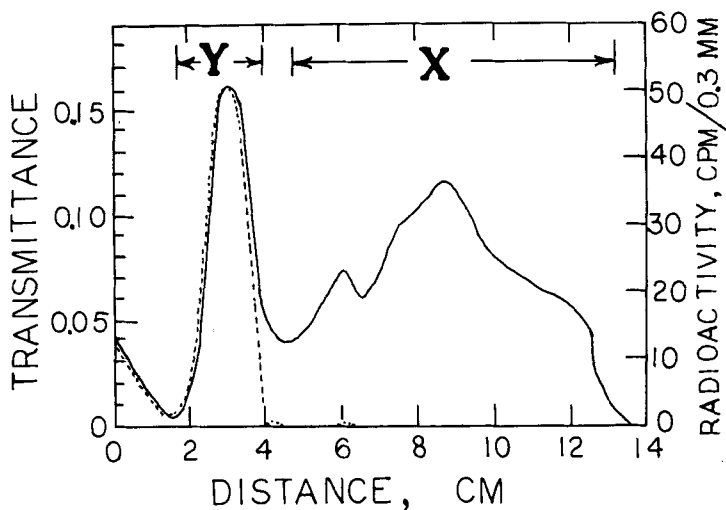

FIG. 4. — Distribution of visible spot (full line) and radioactivity (dotted) over the chromatogram of figure 3, A′.

over the same chart. Sharp coincidence of radioactivity peak with the spot Y in visible chromatogram informs that tritium has been incorporated into the acid only.

Reduction of X to 1-dodecanol-2,3-^{3}H, ^{3}H (IV) was done by the Nystrom's method [10] with some modifications. In figure 5 are shown the infrared absorption spectra of the acid (X) and the alcohol (IV). Each of them has proven to be identical to that for pure samples of non-radioactive acid and alcohol, respectively. It can be seen in the figure that the upper spectrum shows no absorption due to C=O stretching which appears in the lower one.
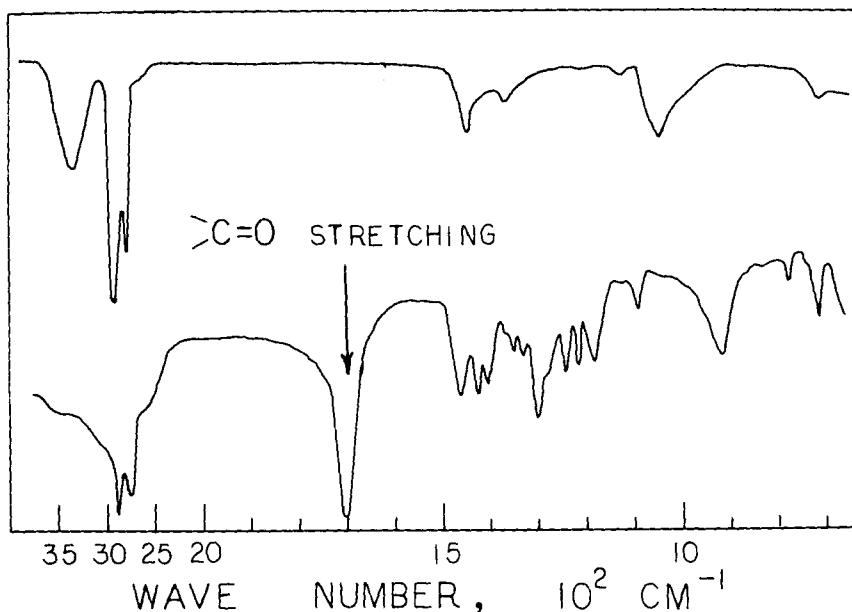

FIG. 5. — Infrared spectra of dodecenoic-2,3-^{3}H, ^{3}H acid (lower) and dodecanol-2,3-^{3}H, ^{3}H.

Gaschromatogram of the product IV is shown in figure 6 in which the main peak has been identified as due to 1-dodecanol. Precise investigations informed that the product contained no impurities in higher temperature region, but trace (less than 5% in the total) amounts of impurities in lower region. One of them was identified as 1-decanol, but other(s) has remained unidentified to any compounds so far available. All these impurities seemed to have existed in the starting material VI, since the same charts were obtained for non-radioactive 1-dodecanol (VI) anf for a LiAlH$_4$-reduced product of non-radioactive dodecanoic acid (VII). Thus, the main part in figure 6 was taken by preparative gaschromatography to obtain a highly pure sample of 1-dodecanol-2,3-^{3}H, ^{3}H.

Sulfation leading to sodium dodecyl-2,3-^{3}H, ^{3}H sulfate (V) was taken mainly after Dreger et al. [11]. After synthesizing the product V, non-radioactive

1-dodecanol was dissolved into an ethanol solution of V and, after evaporating the solvent, removed by extraction with ether. This was repeated twice for lowering the specific activity of IV which might have remained in the crude
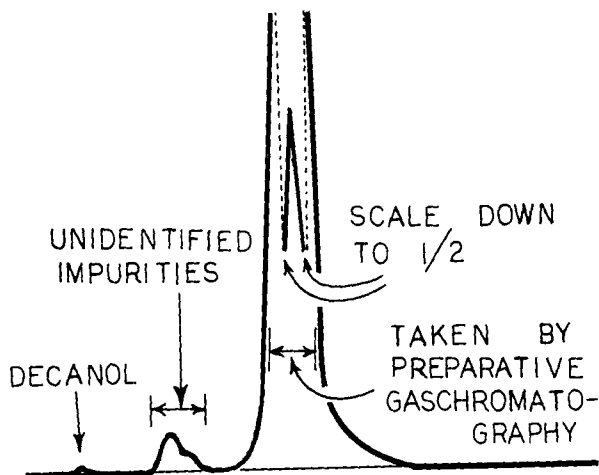

FIG. 6. — Gaschromatogram of 1-dodecanol-2,3-^{3}H, ^{3}H.
Stationary phase, 1.6 m Carbowax 20 M at 228 °C.
Carrier gas, 50 ml/min nitrogen plus 30 ml/min hydrogen detection, flame ionization.
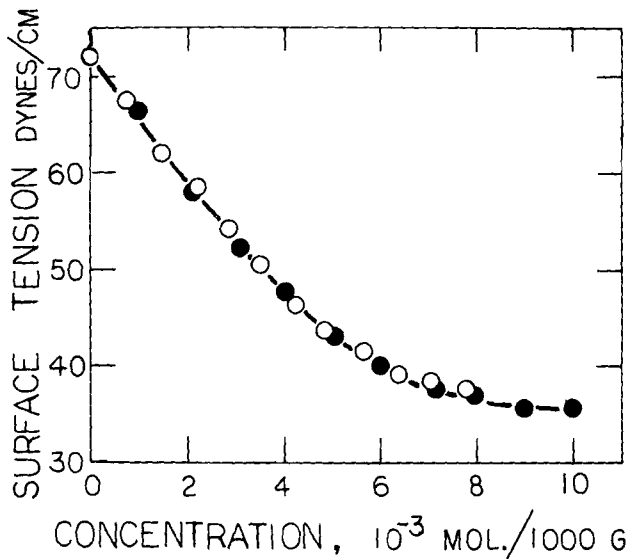

FIG. 7. — Concentration dependence of surface tension of tritiated (○) and non-radioactive
(●) SDS solutions at 25 ± 0.5 °C.
Surface tension determined by drop weight method.

product. Then the ordinary purification was achieved for surface-chemically pure sample of V. Figure 7 gives the concentration-dependence of surface tension for the product V in comparison with that for an extremely pure sample of non-radioactive SDS. Coincidence for two samples which show no depressions in the curve is evidently the indication of high purity of the product V [12].

It is thus concluded that the radiochemical and surface-chemical purities for the product V are high enough to be used for the measurement in high precision of adsorbed amounts of sodium dodecyl sulfate at its solution surfaces. Also, purity of the product IV, 1-dodecanol-2,3-^{3}H, ^{3}H, is confirmed to be as high as that of the product V.

EXPERIMENTAL

Dodecanoic acid (VII)

A solution of 50 g of potassium perchromate in 470 g of 15% sulfuric acid was added drop by drop to 50 g of 1-dodecanol (VI), which had shown the boiling point of 141-142 °C/11 mm Hg upon the fractional distillation using a column of 70 H. E. T. P. After boiling the mixture for 1 hr., excess perchromate was decomposed with sodium sulfite and an ethereal extract was washed successively with hydrochloric acid, sodium bicarbonate and water. Fractional distillations of the residue from the evaporated ethereal extract gave an equimolar mixture of acid and alcohol upon saponification with a solution of sodium hydroxide. The combined acid was purified by repeated crystallizations of its lead salt and finally by fractional distillations of acid boiling at 156-157 °C/4 mm Hg. Yield 55%, m.p. 44.5 °C.

2-dodecenoic acid (IX)

To a mixture of 83 g of VII and 4.5 g of phosphorus trichloride, 80 g of dried bromine was added gradually and the mixture was gently refluxed for 13 hrs. After decomposing the excess bromine with sodium sulfite, reaction mixture was made acidic. Ethereal extract was washed successively with dilute hydro-chloric acid, sodium bicarbonate and water and, after drying with magnesium sulfate, it was evaporated to dryness to give 100 g of crude bromododecanoic acid (VIII).

To the above product, VIII, 80 g of fine powders of dried potassium iodide and 700 ml of absolute ethanol were added, and the mixture was refluxed for 6 hrs. and subsequently for 3 hrs. with 75 g of potassium hydroxide. The reaction mixture was made acidic and extracted with ether. The extract was washed, dehydrated with sodium sulfate, decolorized with charcoal, and kept for standing in a refrigerator for about 48 hrs. to crystallize insoluble 2-hydroxydodecanoic acid. The dried residue from the filtrate was reacted with 9 g of lead acetate in 500 ml of boiling 95% ethanol and the whole mixture was cooled gradually to

15° C, at which it was kept for overnight to separate lead salt of unreacted VII. The evaporated residue from the filtrate was boiled with hydrochloric acid (1:1 in volume) and the oily layer was extracted with ether. The solvent, after washing, was evaporated to give 48 g of crude 2-dodecenoic acid. The crude acid was dissolved in 120 ml of absolute ethanol and reacted with 6 g of lithium hydroxide in 120 ml of water. The mixture was kept for standing for overnight to obtain 30 g of crystalline lithium 2-dodecenoate which then was recrystallized from 80% ethanol. After it was decomposed with boiling hydrochloric acid, liberated acid was extracted with ether and the extract was washed, dried, and finally evaporated to dryness. The residue was distilled several times to give finally b.p. 145.0-146.0 °C/2.5 mm Hg. Yield, 12.3 g or 15% of theoretical based on VII.

Dodecanoic-2,3-^{3}H, ^{3}H acid (X)

A suspension of 0.5 g of U-Ni in 10 ml of dioxane in the flask E of figure 1 was saturated with hydrogen gas. A portion of 1.66 g of IX was then added to the frozen suspension and, after repeated flushing and final evacuation of the whole vacuum line, the ampoule A was broken to introduce tritium gas into the graduated Toepler, D, to collect all aliquots of hydrogen gas which was used for washing the broken ampoule. The mixture (ca. 5 ml at 1 atm, room temperature) of tritium and hydrogen was brought into the molten reaction mixture under stirring. When absorption of mixed gas was completed, additional aliquot of 5-10 ml of supplemental hydrogen was brought successively into E via A and D. This was done repeatedly until the reaction mixture in E absorbed no more hydrogen, as shown in figure 2.

Hydrogenated product was acidified, and the ethereal extract was washed and evaporated to give 1.9 g of residue which was then dissolved into 50 ml of methanol. The solution was allowed to stand for a few hours and evaporated to dryness. This was repeated seven times to lower specific activity of labile tritium which might have remained in the residue. Twice recrystallizations gave eventually 1.2 g of dodecanoic-2,3-^{3}H, ^{3}H acid which, as has been mentioned before, was identified as pure compound, m.p. 42.8-43.1 °C.

1-Dodecanol-2,3-^{3}H, ^{3}H (IV)

To an ice-cooled mixture of 35 ml of ether and 1.3 g of lithium aluminum hydride, a solution of 2.7 g of X in 25 ml ether was added under vigorous stirring during 3 hrs. while temperature was kept below 5 °C. The mixture was kept for standing at the same temperature for over 40 min. and decomposed with 10 ml of water, followed by ca. 20 ml of 10% sulfuric acid. Ethereal extract was washed repeatedly, dried with magnesium sulfate, and evaporated to dryness. The residue was distilled fractionally three times to give 2.28 g of b.p. 96-99 °C/1.5 mm Hg portion. The preparative gaschromatography of the distillate gave a pure sample (see fig. 6) of IV, 1 g in amount or 43.8% to theoretical.

Sodium dodecyl-2,3-^3H, ^3H sulfate (V)

A portion of 1.1 g of chlorosulfonic acid was added in the course of 3 hrs. to a solution of 0.76 g of IV in 1 ml of dried ether, while the temperature was kept for standing at 23.5 ±0,5 °C. The reaction mixture was kept for standing at 19-23 °C for 20 hrs. and made slightly alkaline (pH 10.5-11) by the addition of 1.16 g of sodium hydroxide under deep cooling. It was then dehydrated by the addition of 35 ml of 1-butanol and subsequent evaporation at 50-60 °C in vacuo. This was repeated three times to obtain completely dried product, which then was treated with 50 ml of absolute ethanol at 75 °C. The insoluble sodium sulfate was filtered and washed with hot ethanol. Combined filtrate was evaporated in vacuo to give 1.2 g of crude V, which was purified in Soxhlet extractor for removing unreacted IV with unhydrous ether.

The product thus obtained was dissolved in absolute ethanol containing 6 mg of non-radioactive dodecanol for isotopic dilution. The solution was evaporated to dryness and the residue, after removing dodecanol with ether in Soxhlet extractor, was recrystallized from ethanol. This procedure comprising dissolution, isotopic dilution, extraction and recrystallization was repeated again to obtain finally 0.735 g of V pure enough to show no depression in its surface tension-concentration curve, as is seen in figure 7.

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