Liquid Tritioammonia as a Tritiating Agent

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Summary

Tritioammonia, 10 Ci/g, tritiated piperidine, 5-hydroxytryptophan, glutamine, and asparagine by refluxing at $-35°$ C. Within 1.5 to 6 hours the specific activities reached 0.02, 0.775, 1.24 and 2.48 mCi/mmole, purified. Cyclooctatetraene was not exchange-labeled under these conditions. For liquid-scintillation counting of tritioammonia, ortho-bromobenzoic acid in toluene-dioxan served well. Autoradiolysis was no problem.

Introduction.

A. I. Shatenshtein terminates his article "Hydrogen Isotope Exchange Reactions of Organic Compounds in Liquid Ammonia" with the conclusion : "Hydrogen exchange reactions in liquid ammonia can be used to prepare many organic compounds labelled with tritium or deuterium" [1]. While ample documentation for the validity of this statement is presented with regard to deuteration, experimental support for the claimed tritiation has been lacking. E. A. Evans' thorough and extensive survey on "Tritium and its Compounds" [2], which appeared three years after Shatenshtein's article, does not mention any basic media among liquids suitable for preparation of tritiated compounds by isotope exchange.

In a research proposal to Euratom, W. Hafferl [3] developed the hypothesis that olefinic and aromatic C-H bonds should exchange hydrogen with liquid tritioammonia in the presence of alkali amide, while certain chemically activated methylene groups might take up some tritium already from pure boiling tritioammonia. Ordinary paraffinic C-H bonds were not expected to react with liquid ammonia even in the presence of alkali amides; boiling tritio-ethylenediamine containing catalysts was suggested as an isotope-exchange medium that might be used for this purpose.

SCOPE.

From a limited list of compounds, five were selected to check the hypo-thesis, which was largely founded on intuition. For up to now, no theoretical concept has been adequately developed to support quantitative reasoning on the hydrogen exchange under self-irradiation. Since Tserevitinov-active hydrogen positions merited no attention here, three paraffinic compounds (piperidine, asparagine, glutamine), one cycloolefin (cyclooctatetraene, "COT" for short), and one (hetero-)aromatic substance (5-hydroxytryptophane) were thought to represent the mentioned types of bonds best within the given framework. The relative ease of purification was also considered for each substance.

The loop-shaped piperidine molecule is constructed of five ordinary methylene groups and one secondary amino function, which permits an analytically sharp separation and purification by precipitation of nickel pentamethylenedithiocarbamate. COT consists of eight identical olefinic methyne functions; it can be purified as a silver nitrate complex [4]. Both aromatic and heteroaromatic C-H bonds occur in 5-hydroxytryptophane, in addition to the aliphatic α-aminocarboxylic acid unit, which is also present in asparagine and glutamine. The indole-position 4 can probably be oxidized with nitrosodisulfonate [5], and degradation of the aliphatic chain has been reported long ago [6]. The relative isotope-exchange rates should manifest themselves in the respective molar radioactivities of the individual derivatives ("fix" H-atoms in bold-face) :
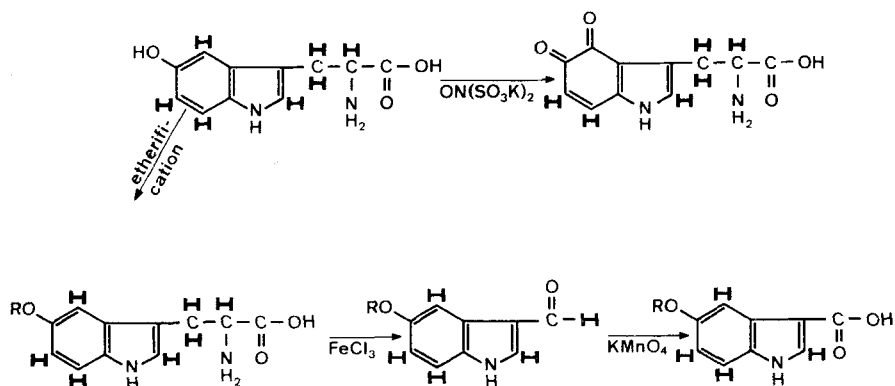
Before taking up the tritiation experiments, the practicability of working with highly tritiated ammonia had to be established. Since the soft β-radiation of tritium (18 keV max.) poses no shielding problems even at 100 % enrich-ment (= 87 Ci/mmole), the autoradiolysis rate of tritioammonia remained the sole technical factor dictating the ceiling of the practicable specific activity,

if any. No pertinent data had been found at the outset of this work. After completion of our measurements, A. H. Zeltmann [7] kindly supplied his results in detail, which had been presented as a paper to the American Chemical Society. Although the manufacturer of our tritioammonia had estimated that specific activities substantially above the 10 Ci/g level supplied should cause the liquid compound to disintegrate inconveniently fast, we found a maximum autoradiolysis rate of 186 ppm/day just above its melting point. When stored at room temperature as a gas of about 0.3 atm., the same material decomposed at a rate of merely 37 $\pm$ 8 ppm/day.

RESULTS AND DISCUSSION.

Although A. H. Zeltmann and A. E. Florin studied a grade of tritio-ammonia of 98.8 % ^{3}H-isotope enrichment, their (pressure-independent) decomposition rate, expressed as $d[NT_3]/dt$(hour) $= -0.00233$ $[NT_3]$, is of the same order of magnitude as our values, if the dose rate is taken in account : Compared with pure NT_3 (87 Ci per mmole), our tritioammonia (10 Ci/g $= 170$ mCi/mmole) was exposed to $170/86,000 = 1.98.10^{-3}$ times the radiation that Zeltmann's tritioammonia emitted on itself. The auto-radiolysis rate of $2.33.10^{-3}$ per hour, equal to 56.10^{-3} per day, would correspond to 111 ppm/day, if this dose-rate extrapolation is permitted. The autoradiolysis rates do, however, depend on admixtures, surface qualities of the containment, and possibly other parameters; our values are therefore reported with the understanding that they may be subject to correction.

By 1.5 hours' refluxing in 0.2 % tritioammonia the following specific activities were attained (mCi/mmole) : COT 0; piperidine 0.02; 5-hydroxy-tryptophane 0.775; glutamine 1.24; asparagine 2.47. The chemical yields were practically quantitative for the crude materials (no perceptible decomposition). While these values are low compared with activities of commonly known tritiated compounds, they should be judged with due consideration for the purpose of this investigation : Merely the feasibility of tritiation in liquid tritioammonia was to be checked against the largely intuitive working hypo-thesis about the relative exchange mobility of paraffinic, olefinic, and (hetero)-aromatic hydrogen atoms. No attempt has been made to optimize the reaction conditions.

While the paucity of experimental data does not warrant elaborating on the results in detail, two observations deserve attention, because they contradict the forecast based on the provisional hypothesis :

1. Liquid tritioammonia alone does introduce tritium in paraffinic C-H bonds reasonably fast.
2. The olefinic COT undergoes no substitutive tritiation under the same conditions, i.e., the olefinic C-H bond of COT is less prone to isotope exchange than the paraffinic C-H bonds of, e.g., piperidine.

Tritiation of the piperidine must have taken place in a "fix" position, since the radioactive base was reclaimed after passing through the following cycle :
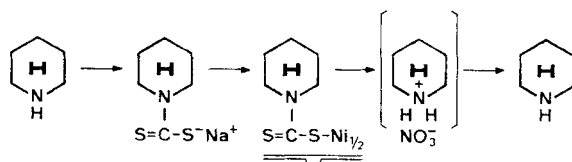
Many questions have remained open, some of which may arouse curiosity predominantly among practitioners of the art of labelling substances, while other problems should appeal more to theoretically-oriented researchers. Since the hydrogen-isotope exchange in liquid tritioammonia promises to be more specific and less harsh than the gas-exchange tritiations [8], the new labelling method may help to fill the gap between the technically excellent but aimless Wilzbach exchange and the comparatively expensive synthetic labelling procedures. Conceivably, liquid tritioammonia may be only one of several mild tritiants still to be tried [3], especially for isotope-exchange labelling of sensitive natural products, which cannot be tagged by other methods.
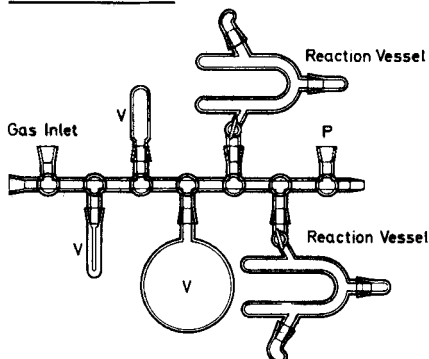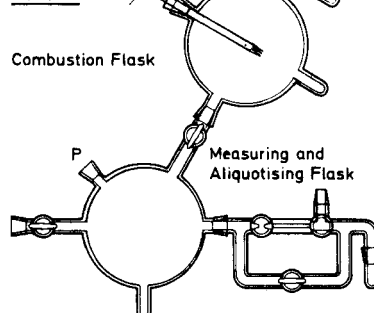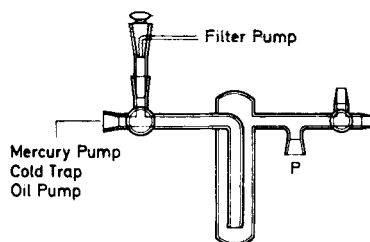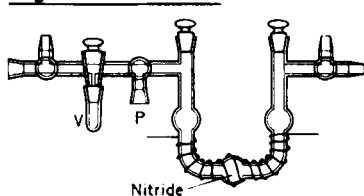
Studies of reaction mechanisms and reaction kinetics often involve knowledge of (or search for) individual bond strengths [9]. There are several pertinent aspects, where a well-directed hydrogen exchange in liquid tritiants or the availability of more or less specifically tritiated substances could produce valuable information, e.g. about lock-and-key reactions.

APPARATUS AND PROCEDURES.

The low-leak ($< 10^{-6}$ torr $\cdot$ l $\cdot$ sec^{-1}) all-glass *apparatus* (figure) consisted of three sections, which allowed to be sealed off individually or connected with each other or with the vacuum pump system through a manifold.

The *reaction section* encompassed a 6-1 storage flask for the gaseous ammonia, a calibrated capillary to measure the liquid ammonia volumetrically, and several individual reactors, where tritioammonia could be kept refluxing in liquid contact with the substrate at any desired temperature between $-77°$ C (m.p.) and $-33°$ C (b.p. at 1 atm). Ammonia-insoluble materials were exposed to the tritiant as thin films on glass beads. Ammonia-soluble substances were weighed in the ST 14.5/30 tubes, which served as detachable refluxing containers. The conical cold fingers of the reflux condensers were sparingly charged with dry ice and acetone to obtain a vivid reflux with a minimum of ammonia.

The *analytical section* permitted ammonia and hydrogen to be estimated individually either by barometric measurements of the gases or by liquid-

Reaction Section

Reaction Vessel

V

Gas Inlet P

V

Reaction Vessel

V

Combustion and Measuring Section

Combustion Flask

P

Measuring and Aliquotising Flask

Regeneration Section

V P

Nitride

Filter Pump

Mercury Pump
Cold Trap
Oil Pump

P

P Pirani Gauge
V Storage Flask

scintillation counting of suitable derivatives. Ammonia and hydrogen were easily segregated by fractional distillation and condensation with liquid nitrogen and liquid helium, respectively. The gases were aliquotized by twisting a cup-bore stopcock; one motion back and forth transferred 0.2 ml gas of the total volume (preselectable 1 to 9 l) into the sorption vessel (ammonia) or into the combustion flask (hydrogen). Ammonia was absorbed by *ortho*-bromobenzoic acid in toluene and counted after addition of a little dioxan to solubilize the ammonium *ortho*-bromobenzoate. Hydrogen was burned with excess oxygen and counted as water.

In the *regeneration section* it was possible to convert water to ammonia. Magnesium nitride reacted at room temperature according to

$$6 H_2{}^*O + Mg_3N_2 \longrightarrow 3 Mg(OH^*)_2 + 2 NH_3{}^*$$
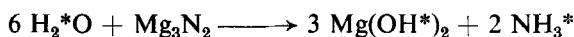
When the hydroxide was heated at 350 to 400° C in the U-tube containing excess magnesium nitride, more ammonia was reclaimed :

$$3 Mg(OH^*)_2 + Mg_3N_2 \longrightarrow 6 MgO + 2 NH_3{}^*$$
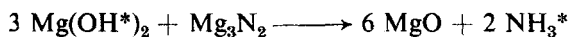
As the apparatus had a leak rate consistently smaller than had been allowed for, and because the autoradiolysis of the tritioammonia proceeded

at an unexpectedly slow pace, the regeneration section turned out to be a mere safety device, which was not actually needed. For the latter reason, hydrogen gas did not have to be aliquotized.

The *tritiation* of the purified substrates was allowed to continue for 1.5 or 6 hours, after the reactor had been evacuated and flooded with enough tritioammonia to keep up a vivid reflux between the tip of the condenser finger and the substrate in the tube underneath, 0.2 to 0.7 ml. For best results, a bath temperature of $-55°$ C to $-40°$ C was maintained for the substrate. The tritioammonia was then volatilized into a liquid-nitrogen cooled receiver within the reaction section. It was later returned to the storage flask after repeated distillation.

Labile activity was largely expelled by three times flooding the tritiated material in the reactor with inactive dry ammonia and evaporating each portion slowly. The remaining mobile activity was leached out with water. To this end water-insoluble material was dissolved in a volatile, water-immiscible liquid, e.g. pentane. The residue was purified by conventional, individually adapted methods. The two liquid substrates, COT and piperidine, were, in addition, purified after conversion to solid derivatives and reconstituted

EXPERIMENTAL DETAILS.

Autoradiolysis of 2 g of tritioammonia (20 Ci) at ambient temperature and 0.3 atm for 19 days gave 247.5 (238.5) nCi of elementary tritium in an aliquot of

$$\frac{0.5 \text{ ml}}{200 \text{ ml}} \cdot \frac{1 \text{ ml}}{100 \text{ ml}} \cdot \frac{7.5 \text{ l}}{8.5 \text{ l}}$$

equal to 11.0 mCi per 20 Ci or 29 ppm per day. In a second experiment, extending over 32 days, 298.9 and 604.5 nCi of hydrogen gas were found in two aliquots of 0.25 and 0.5 ml per 200 ml of a 1 : 100 diluted solution of radioactive water obtained by collecting and catalytically burning 7.5 out of 9.0 l of hydrogen gas in the apparatus with excess oxygen, corresponding to 28.7 (29.0) mCi or 45 ppm/day. The average of both experiments is 37 $\pm$ 8 ppm/day.

The same quantity of tritioammonia was stored as a liquid just above its melting point in a well sealed to the bottom of a round-bottom flask of 1 l volume, forming an integral part of the all-glass apparatus. After 14 days the tritioammonia was evaporated and condensed again with liquid nitrogen. The remaining gas was expanded to 8.5 l, 7.5 l of which was diluted with 9.9 ml (STP) of inactive hydrogen and solidified with liquid helium. The material was fractionally distilled into the liquid-helium cooled well of the combustion flask resembling a Kalberer flask. The residual gas in the apparatus, except for that which was locked up in the 1-l flask with the sealed-on well to contain the bulk of the recondensed ammonia, was collected with another

small quantity of inactive hydrogen in a liquid-helium cooled well and subsequently fractionated like the first bulk of the elemental hydrogen.

The combined hydrogen fractions were burned in the combustion flask with excess oxygen, added in six 100-ml (STP) portions to the hydrogen from the far end of the apparatus to effect a flushing motion. After the third ignition had been triggered by electrically heating the platinum coil, no further signs of a combustion, like a flame or a pressure drop, have been detected.

The combustion product, tritiated water, was absorbed by freshly ignited (650° C) calcium oxide at $-78°$ C. The water was liberated with 5 ml of 10 % hydrochloric acid in a volumetric flask and diluted to 100 ml. A 1-ml sample of a 1 : 200 dilution of the stock solution gave 292,398 Imp/min (dioxan), with 0.1 ml ^{3}H-toluene standard (391 nCi) 342,360 Imp/min (background 165 Imp/min). This corresponds to 45.8 mCi in 7.5 l, 52 mCi in 8.5 l, gas or 186 ppm/d. A repeat investigation after attempts to tritiate COT gave a lower autoradiolysis rate, 52.3 $\pm$ 1 ppm per day, for the liquid ammonia [10].

Of 207 mg COT refluxed for 6 hours, 139.4 mg was retrieved after three extractions of its 2-methylbutane solution with water and standing over calcium chloride. Liquid scintillation counting of 1 ml toluene containing 2.8 mg COT per 100 ml gave 59,630 Imp per minute without and 134,814 Imp/min with 391 nCi ^{3}H-toluene standard (119 Imp/min background) corresponding to 1.175 mCi per mmole.

Since repeat runs gave poorly reproducible specific activities, the aqueous extracts of one experiment were tested for labile activity. The three total volumes of subsequent extracts contained 1572.3, 625.4, and 124.5 μCi, the desiccant 0.66, 0.50, and 0.33 μCi, i.e. only small fractions of the total COT activity.

One radioactive sample was diluted to 1 g with inactive COT and allowed to react in 2.5 ml ligroin with 1 g AgNO$_3$ in 2.5 ml water at 50° C. From the ice-cold mixture, 1.26 g cream-colored 3 AgNO$_3 \cdot$ 2 C$_8$H$_8$ (desiccator-dry) was collected and recrystallized (720 mg) from 20 % aqueous AgNO$_3$. The strongly quenching complex was decomposed in 3 ml water with 600 mg NaCl, and the COT was collected in pentane after steam-distillation (101 mg). Both the COT and the aqueous distillate were inactive.

Piperidine, 170 mg (2 mmoles), was refluxed with 0.2 ml tritioammonia at $-35°$ C for 1.5 hour, flushed three times with inactive dry ammonia, and converted into insoluble nickel pentamethylenedithiocarbamate [11]. The base was reclaimed by dissolving the nickel salt in hot 2 N nitric acid, steaming out the carbon disulfide, and collecting the steam-distillate from the alkalinified nickel solution in 2 drops of 2 N hydrochloric acid. The desiccator-dry (H$_2$SO$_4$ after benzene-ethanol evaporation) piperidinium chloride (75 % yield) gave a liquid-scintillation count of 48,238 Imp/min for 1.4 mg (with background, 119 Imp/min), 129,849 Imp/min after addition of 391 nCi of ^{3}H-toluene standard, corresponding to 230.5 nCi, i.e. 20.02 μCi/mmole or 2 mCi per mole of C-H bond.

Asparagine, glutamine, and 5-hydroxytryptophane, 148.2, 158.2, and 43.7 mg, were refluxed in 0.5 ml tritioammonia, wherein 5-hydroxytryptophane dissolved completely, asparagine and glutamine partly. The purified substances were identified by paper chromatography in butanol-acetic acid-water 1 : 1 : 1 (Glutamine) or 4 : 1 : 1.

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