[Chem. Pharm. Bull. 23(11)2944–2948(1975)]

UDC 546.212.02.04 : 547.853.3'771.02.057 : 547.568.1.02.057

Synthesis of Tritium-labeled Compounds*

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(Received April 28, 1975)

Improved Wilzbach tritiations and tritiations with tritiated water are compared with results obtained by direct syntheses of tritiated compounds. The syntheses of [6-^{3}H]-4-hydroxypyrazolo[3,4-*d*]pyrimidine, [1-^{3}H]-2-amino-1-phenylpropanol and [2,3-^{3}H]-tributyltin benzoate are described.

It has been known for some time that organic compounds are decomposed to a large extent by the Wilzbach tritiation, and some improvements have already been reported. Methods have been developed which use tritiated water for uniform labeling. When performed in the presence of freshly reduced platinum oxide, the so-called "catalytic tritium labeling" has shown hopeful results and has been proved with over 300 substances.[2]

We needed tritium-labeled compounds for biological tests and, in some of these compounds, specific position of labeling was not necessary. Some others were to be used as the starting material for further syntheses, which in some cases were double-labeled compounds, *e.g.*, 2-amino-1-phenyl-propanol[1-^{3}H]. In all these compounds, adequate radioactive purity and absence of easily exchangeable tritium were of greatest importance. The specific activity and the final chemical and radiochemical yield of these compounds had to be high enough for biological testing or further synthesis.

Some improved methods described in past literature for uniform labeling of 4-hydroxypyrazolo[3,4-*d*]pyrimidine and 2-amino-1-phenyl-propanol were examined and compared with the methods we developed (*cf.* Table I, II). The known methods examined were the following three:

TABLE I. Tritium Labeling of 4-Hydroxypyrazolo[2,3-*d*]pyrimidine

Method	Gaseous ^{3}H		^3H_2O		Synthesis
	Wenzel, *et al.*[3]	Gosztonyi[4]	Garnett[2]		
Start (Ci)	10	3	8	8	3
(mg[a])	25	57	70	70	
Reaction time (hr)	300	1	16	16	
Temperature (°C)	−190	20	60	100	
Product (mCi)	0.009	3.4	0.109	0	12
Product (mg)	5	31	37	0	200

a) Amount of inactive 4-hydroxypyrazolo[3,4-*d*]pyrimidine added.

(1) Improved Wilzbach Tritiation[3]: The substance to be labeled must first be adsorbed on active carbon and then treated with gaseous tritium at −190°. This method was tried with 4-hydroxypyrazolo[3,4-*d*]pyrimidine and a product was obtained only after the addition of 50 mg of pure inactive compound. Recrystallization gave 5 mg of a product of acceptable

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* Dedicated to the memory of Prof. Eiji Ochiai.

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2) J.L. Garnett, *Nucleonics*, **1962**, 86; G.E. Calf and J.L. Garnett, *Chem. Commun.*, **1969**, 374.

3) M. Wenzel, *et al.*, Tritiumsymposium Wien, TTS/66 (1961). International Atomic Energy Agency, Wien; M. Wenzel, P.E. Schulze, and H. Wollenberg, *Naturwissenschaften.* **44**, 515 (1962).

Table II. Tritium Labeling of 2-Amino-1-phenyl-1-propanol

	Treatment with ^3H_2O (1 Ci/ml)			Synthesis
Start (Ci)	5	5	3.5	0.42
Start (mg)	530	1300	250	
Reaction time (hr)	60	60	140	
Amount of PtO (mg)	125	140	300	
Temperature (°C)	70	80	120	
Product (mCi)	9.4	22	—	54
Product (mg)	300	630	—	321

chemical purity but the radiochemical impurities were extensive (*cf.* Fig. 1).

(2) Action of Gaseous Tritium under Influence of Microwaves[4]: By the use of this method, radioactive 1-hydroxypyrazolo[3,4-*d*]pyrimidine was isolated without dilution with a cold compound. At first, the specific activity seemed satisfactory but radioscanning thin-layer chromatogram (TLC) has shown that most of this activity originated from impurities, and the active product was situated mainly at the starting point, far from the *Rf* value of the compound itself (*cf.* Fig. 1).

(3) Catalytic Tritium-labeling with Tritiated Water[2]: 4-Hydroxypyrazolo[3,4-*d*]pyrimidine and 2-amino-1-phenyl-propanol were treated with tritiated water under various conditions but we could not get satisfactory results from any of the conditions (*cf.* Table I). The specific activity of 4-hydroxypyrazolo[3,4-*d*]pyrimidine was not high enough when treated under mild conditions and extended treatment afforded a substance contaminated with a large amount of impurities, its purification being impossible.

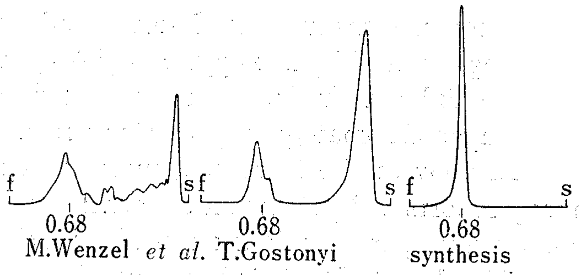

Fig. 1. Radioactivity Peaks in Tritiated 4-Hydroxypyrazolo[3,4-*d*]pyrimidine on Radioscanned Thin-Layer Chromatograms

s=start, f=front
plate: Silicagel F_{254} pure inactive compound: *Rf* 0.68
solvent system: BuOH: propionic acid: H_2O=70 : 30 : 15

Treatment of 2-amino-1-phenyl-propanol with tritiated water afforded a chemically pure product whose activity was not reduced by repeated recrystallization. This product was treated alternately with acids and bases till its specific activity remained constant, indicating that there would be no nitrogen-bonded tritium in the amino group. Nevertheless, all the radioactivity was lost on subsequent Mannich synthesis. If all the activity disappeared in such a reaction, the tritium must have been bonded to a position which participated in the Mannich synthesis, as its condition is much milder than that used during the purification treatment. Further experiments seem necessary to decide how far tritium in the amino group itself might be stable against exchange under basic and acidic conditions, or if by such a labeling with tritiated water tritium is introduced into a position which would participated in the Mannich synthesis, whose mechanism is not well understood at present.

Since none of the above-described methods for uniform labeling gave us the products of the qualities we needed, we decided to synthesize all the necessary compounds.

For the synthesis of 4-hydroxypyrazolo[3,4-*d*]pyrimidine[6-^{3}H], we adopted the method of Robins[5] who described the synthesis of unlabeled compound on a large scale, as it allows labeling in the last step. There was no difficulty in carrying out this synthesis on a small laboratory scale. 3-Aminopyrazole-4-carboxamide sulfate was synthesized from monochloroacetic acid and condensed with 2 mol instead of 15 mol of formamide[1-^{3}H).

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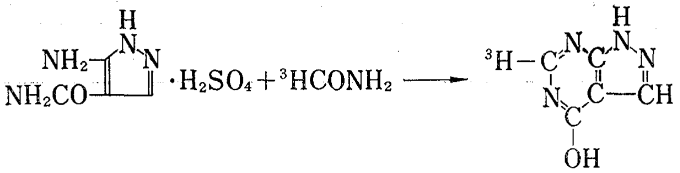
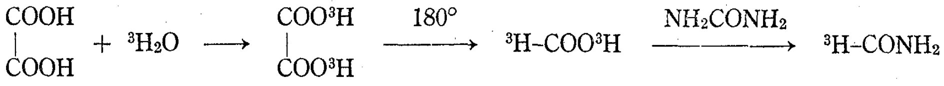
4) T. Gosztonyi, Actiebolaget Atomenergi (Stockholm), AE-345 (1969).
5) R.K. Robins, *J. Am. Chem. Soc.*, **78**, 784 (1956).



Formamide[1-^{3}H] was synthesized according to the method for the synthesis of deuterated formic acid,[6] combined with the preparation of ^{13}C-formamide from ^{13}C-formic acid.[7] The hydrogen atoms in the carboxyl groups of oxalic acid were first exchanged with tritium by treatment with tritiated water, then a tritium-carbon bond was formed by decarboxylation of this tritiated oxalic acid to form ^{3}H-formic acid, and this was derived to formamide[1-^{3}H] by treatment with urea.



$$\begin{matrix}COOH\\ |\\ COOH\end{matrix} + {}^3H_2O \longrightarrow \begin{matrix}COO^3H\\ |\\ COO^3H\end{matrix} \xrightarrow{180°} {}^3H\text{-}COO^3H \xrightarrow{NH_2CONH_2} {}^3H\text{-}CONH_2$$

After condensation of ^{3}H-formamide with 3-amino-4-pyrazolecarboxamide sulfate, the resulting crude 4-hydroxypyrazolo[3,4-*d*]pyrimidine[6-^{3}H] was recrystallized, and its radioactivity did not diminish after repeated recrystallizations from acidic and basic solvents. Thin-layer chromatography of this product proved its radioactive purity (*cf.* Fig. 1).

In view of the increasing interest in norephedrine and norpseudoephedrine, there are many reports and patents on the synthesis and separation of various stereoisomers of 2-amino-1-phenyl-propanol (*cf.* Beilstein's Handbuch der Organischem Chemie, Vol. 13, Syst. No. 1855: *E*^{1}, 253, *E*^{2}, 370, *E*^{3}, 1717) but none of these methods are suitable for the synthesis of tritium-labeled compounds except the direct hydrogenation of 2-amino-1-phenyl-propiophenone.[8] As we had in mind to elucidate the results we obtained from the Mannich synthesis with the uniformly labeled compound, labeled-2-amino-1-phenyl-propanol was needed where the tritiation of the amino group would be a minimum. Therefore, the reported hydrogenation of 2-isonitrosopropiophenone[9] had to be avoided. For the same reason, methods like that of Sah[10] in which the aminoketones acylated in the amino group were hydrogenated. The reductive cleavage of the acyl group would also introduce tritium in the amino group, and tritium would be wasted by the formation of unnecessary by-products. Further, radioactive by-products might be formed in such a competing side reaction and separation of the by-products would be very difficult, especially if tritium diluted with hydrogen is used. In view of the great isotope effect between tritium and hydrogen, tritiation might occur predominantly for the formation of by-products and in the amino group.

Although reports on the hydrogenation of 2-aminopropiophenone are not extensive, no trouble arose by the reductive tritiation with a palladium catalyst. 2-Aminopropiophenone was prepared from 2-bromopropiophenone and phthalimide.[11] Separation of optical isomers was not necessary for our objective. The Mannich synthesis was carried out on this 2-amino-1-phenyl-propanol[1-^{3}H].[12] The radiochemical yield was 17% below the chemical yield, contrary to the reaction with the uniformly labeled compound. Therefore, 83% of tritium

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6) R.C. Herman and S.V.Z. Williams, *J. Chem. Phys.*, **8**, 447 (1940).
7) E. Cherbuliez and F. Landolt, *Helv. Chim. Acta*, **29**, 1438 (1946); H.R.V. Arnstein and R. Bentley, *J. Chem. Soc.*, 3509 (1951).
8) A. Eberhard, *Arch. Pharm.*, **255**, 142 (1917).
9) P. Rabe, *Ber. Dtsch. Chem. Ges.*, **45**, 2166 (1912); H. Adkins and H.T. Cramer, *J. Am. Chem. Soc.*, **52**, 4349 (1930).
10) P.T.T. Sah, *Ber. Dtsch. Chem. Ges.*, **71**, 2300 (1938).
11) Ch. Schmidt, *Ber. Dtsch. Chem. Ges.*, **22**, 3251 (1889).
12) F.A. Drahowzal and F. Wiesinger, *Liebigs Ann. Chem.*, **734**, 199 (1970).

was definitely in the position different from that after uniform labeling, and the tritium labeling must have been at the carbon atom bearing the hydroxyl group. It may therefore be concluded that this position does not interfere in the Mannich synthesis.

Although the synthesis of inactive[13] as well as radioactive tributyltin-113 benzoate[14] is known, preparation of butyl[2.3-^{3}H]tin benzoate had to be extensively modified. First, 1-butanol[2,3-^{3}H] was prepared from a solution of 2-buten-1-ol in 1-butanol by its treatment with tritium which was diluted with hydrogen. Freshly reduced platinum oxide was used as a catalyst. A gas chromatogram of the product from this reaction using the inactive compound proved that all of 2-buten-1-ol had been converted to 1-butanol. Therefore, tributyl[2,3-^{3}H]tin benzoate was prepared from 1-butanol[2,3-^{3}H] by the following route (where *Bu indicates butyl[2,3-^{3}H] group):

$$\text{*Bu-OH} \xrightarrow[\text{pyridine}]{\text{PBr}_3} \text{*Bu-Br} \xrightarrow{\text{Mg, ether}} \text{*Bu-MgBr} \xrightarrow{\text{SnCl}_4} (\text{*Bu})_4\text{Sn} \xrightarrow[\text{heating}]{\text{SnCl}_4}$$

$$(\text{*Bu})_3\text{SnCl} \xrightarrow{\text{CH}_3\text{ONa}} (\text{*Bu})_3\text{SnOCH}_3 \xrightarrow{\text{C}_6\text{H}_5\text{COOH}} (\text{*Bu})_3\text{SnOCO-C}_6\text{H}_5$$

The Grignard compound proved to be better than tributylaluminum[15] in this synthesis with tritium-labeled alkyls. Direct formation of the benzoate from tributyltin chloride[14] was accompanied some hexabutylditin as radioactive contaminant. Saponification with aqueous potassium or sodium hydroxide proved to be not very satisfactory. In a later experiment, tributyltin chloride was converted to tributyltin hydroxide or oxide, and then reacted with sodium benzoate to yield tributyltin benzoate. When the chlorine atom was substituted with a methoxyl group,[16] the tributyltin methoxide thereby formed afforded a very pure benzoate in a high yield.

Experimental

In all the experiments with gaseous tritium, a sealed tube containing tritium was introduced into a closed apparatus, the tube was opened by a special equipment, and gaseous tritium was transferred to a gas buret by the aid of Hg in the usual way. Side reactions during the synthesis from decomposition by radiation can be prevented by dilution of tritium with hydrogen. Uniform labeling with gaseous tritium was discouraging in the case of 4-hydroxypyrazolo[3,4-*d*]pyrimidine. For catalytic labeling with tritiated water, PtO was always reduced with hydrogen.

4-Hydroxypyrazolo[3,4-*d*]pyrimidine[6-^{3}H]——After drying *in vacuum* at 100°, 1.5 g (16.7 mmol.) of oxalic acid was treated with 1.3 ml of $^{3}H_2O$ (3 Ci) at 50° for 15 hr, by which only a small amount of the substance dissolved. $^{3}H_2O$ was evaporated *in vacuum* and the residue was heated at 180° to effect decarboxylation of oxalic acid to ^{3}H-formic acid to which 1.3 g (21.7 mmol.) of urea was added and the mixture was heated at 155°. Formamide[1-^{3}H] was formed and it distilled on heating at 150° *in vacuum.*

A mixture of 135 mg (3 mmol) of this formamide[1-^{3}H] and 355 mg (1.5 mmol) of 3-amino-4-pyrazolecarboxamide sulfate was heated at 180—190° for 45 min, the mixture was cooled, and poured into water. The separated crystalline product was recrystallized from MeOH–HCl and then from MeOH–KOH to 200 mg of 4-hydroxypyrazolo[3,4-*d*]pyrimidine[6-^{3}H] with radioactivity of 12 mCi (8.16 mCi/mmol). Its purity was proved by radioscanning of the TLC on silica gel F_{254} (E. Merck) and its *Rf* of 0.68 with the solvent system of BuOH: propionic acid: H_2O (70: 30: 15) was identical with that of the inactive pure compound (*cf.* Fig. 1).

Uniformly Tritium-labeled 2-Amino-1-phenylpropanol——The free base and its hydrochloride were treated with $^{3}H_2O$ in the presence of freshly reduced PtO. In both cases, tritiated compounds were isolated and recrystallized from iso-PrOH. Although these products were treated with alkali and acid until the radioactivity remained constant, almost all the activity was lost in the following Mannich reaction, and

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13) J. Valade and M. Pereyre, *C.R. Acad. Sci.*, **254**, 3693 (1962).

14) P. Ph. H.L. Otto, H.M.J.C. Creeners, and J.G.A. Luijten, *J. Labelled Compd.*, **2**, 339 (1966).

15) W.J. Jones, D.P. Evans, T. Gulwell, and D.C. Griffiths, *J. Chem. Soc.*, **1935**, 39. W.P. Neumann, *Liebigs Ann. Chem.*, **653**, 157 (1962).

16) D.L. Allestone and A.G. Davies, *J. Chem. Soc.*, **1962**, 2053.

930 mg (6.14 mmol) of 2-amino-1-phenylpropanol[U-^{3}H] of 31.4 mCi gave a chemical yield of 30% but the tritium yield was 0.6 mCi, *i.e.*, 2%.

2-Amino-1-phenyl-1-propanol[1-^{3}H]——To a solution of 355 mg (2.37 mmol.) of 2-aminopropiophenone, prepared from propiophenone,[11] dissolved in 2.5 ml of dimethylformamide, 85 mg of Pd-C was added as a catalyst, O_2 was removed completely by flushing with N_2 three times, and the mixture was hydrogenated with 54 ml (2.4 mmol.) of H_2 containing 420 mCi of $^3H_2$. After removal of the catalyst, formamide was distilled off *in vacuum*, the residue was dissolved in absolute EtOH, HCl was added, and the hydrochloride was precipitated by the addition of ether. This procedure of dissolving and precipitating was repeated twice more to remove most of the exchangeable ^{3}H. The yield of the product so obtained, 321 mg (2.12 mmol.), represents a chemical yield of 90%, and the radioactivity of 54 mCi equals 13% yield (25 mCi/mmol.). In the following Mannich reaction, the chemical yield was 70% and the tritium yield was 56.8% or 28.5 mCi.

Tributyl[2,3-^{3}H]tin Benzoate——To a solution of 288 mg (4 mmol.) of 2-buten-1-ol dissolved in 2.5 g (34 mmol.) of 1-butanol, 15 mg (0.07 mmol.) PtO was added and the solution was treated with 10 Ci of gaseous tritium diluted with hydrogen. After consumption of 90 ml (4.01 mmol.) of ^{3}H, the remaining gas was transferred to a gas buret. This mixture was cooled in ice, 600 mg (7.6 mmol.) of pyridine and 10 g (38 mmol.) of PBr_3 were added, and the whole was refluxed for 4 hr. This mixture was poured into ice water and 4.2 g (30 mmol., 79% chemical yield) of butyl[2,3-^{3}H] bromide, with radioactivity of 4.3 Ci (143.3 mCi/mmol.), was isolated. This labeled compound was diluted with 23.2 g (170 mmol.) of inactive BuBr.

The Grignard compound was formed from 5 g (206 mmol.) of Mg turnings by boiling in 60 ml of ether for 1 hr. A solution of 13.2 g (51 mmol.) of $SnCl_4$ in 25 ml of benzene was diluted with 30 ml of ether and added slowly into the Grignard compound at 0°. This mixture was stirred at room tsmperature for 14 hr, at 50° for 1 hr, cooled, and poured into a mixture of ice and HCl. By fractional distillation, the dried benzene extract gave 16 g (46 mmol., 92% chemical yield) of tetrabutyl(2,3-^{3}H)tin, with radioactivity of 4.0 Ci (87 mCi/mmol.) or 95% ^{3}H yield, n_D^{20} 1.4736 (reported[15]) 1.4730.

For the disproportionation, 3.9 g (15.0 mmol.) of $SnCl_4$ was added to 16 g (46 mmol.) Bu_4Sn and the mixture was heated at 220° for 1 hr, at 215° for 3 hr, and finally heated briefly at 230°. By distillation, 17 g (52 mmol., 85% chemical yield) of tributyl[2,3-^{3}H]tin chloride was separated from the solid residue, with a radioactivity of 3.4 Ci (65 mCi/mmol.) or 85% ^{3}H yield. This was added to a suspension of 52.3 mmol of MeONa in benzene, prepared from 1.2 g of Na, and the mixture was warmed gently with stirring. The resulting NaCl was separated, benzene was evaporated *in vacuum*, and distillation of the residue *in vacuum* gave 12.7 g (39.6 mmol., 76% chemical yield) of tributyl[2,3-^{3}H]tin methoxide, with radioactivity of 2.55 Ci (64 mCi/mmol.) or 75% ^{3}H yield.

This labeled tributyltin methoxide was dissolved in benzene, 4.8 g (39 mmol) of BzOH was added, benzene was distilled off *in vacuum*, and the residue was heated at 60° for 1 hr. Distillation of the residue yielded 15 g (36 mmol., 91% chemical yield) of tributyl[2,3-^{3}H]tin benzoate, bp 168°/1 Torr (reported[13]) bp 166—168°/1 Torr), with radioactivity of 2.29 Ci (63.6 mCi/nmol.) or 89.8% ^{3}H yield, which would be 53% ^{3}H yield calculated from butyl[2,3-^{3}H] bromide. n_D^{20} 1.5157 (reported[13]) 1.5157). A radioscanning TLC proved its radiochemical purity[17]) with *Rf* 0.8 on silica gel G (E. Merck), developed with (iso-Pr)$_2$O+1.5% AcOH.

This short paper was written in memorium of Prof. Dr. Eiji Ochiai. With his death, the world lost a great scientist but, I myself have lost a wonderful friend whom I shall never forget. (F.A. Drahowzal).

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17) G. Neubert, *Z. Anal. Chem.*, 203, 265 (1964).