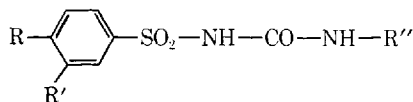


Preparation of Tritium-Labeled Compounds I. Series of Sulfonylurea Hypoglycemic Agents by Exchange with Tritium Gas

By RICHARD C. THOMAS and GEORGE J. IKEDA

A series of tritium-labeled sulfonylurea hypoglycemic agents was prepared by the tritium-gas exposure method. Sodium salts of the sulfonylureas were found to incorporate stably bound tritium more effectively than did the corresponding free compounds. Exposure to tritium gas in the presence of an electrical discharge was also found to be an effective method for preparing tritium-labeled sulfonylureas.

AS PART of a program to develop improved hypoglycemic agents of the sulfonylurea class (I), it became necessary to label a number of these com-



pounds with tritium for use in studying their absorption, distribution, metabolism, and excretion in humans. The tritium-gas exchange method of Wilzbach (1) was chosen because of the relatively low cost of tritium gas and the need for labeling a large number of sulfonylureas, not all of which would necessarily prove of sufficient clinical interest for continued study. An advantage of the Wilzbach method is that a large number of compounds can be exposed to tritium with minimal cost and little investment in time. If a particular compound proves of sufficient interest, the more difficult task of purification can then be undertaken without delay. Thus, in the present case, only 7 of a series of 10 sulfonylureas that had been exposed to tritium were actually purified.

Preparing this series of tritium-labeled sulfonylureas offered an opportunity to study not only the feasibility of labeling this type of compound by tritium-gas exposure but the effects of several variables on their incorporation of tritium. Thus, 15 sulfonylurea samples, representing 7 structure types within the series, were tritiated and purified. Five compounds were exposed to tritium, both as the free sulfonylurea and as the corresponding sodium salt. Two additional sulfonylureas were tritiated, both by conventional tritium-gas exposure and by exposure in the presence of an electrical discharge.

Although exposure of a compound to tritium is relatively inexpensive and easy, subsequent purification of the compound to a state of radiochemical purity can be extremely time consuming and difficult. Trace impurities of very high specific activity are often formed by reduction of an unsaturated bond or by radiation-induced reactions, such as fragmentation, isomerization, and polymerization (1-4). Such impurities, although present in only trace amounts by weight, may contain the bulk of the tritium in the crude sample. These impurities are often difficult to remove, and great care must be taken to establish the radiochemical purity of the final product. Attainment of constant specific activity following recrystallization from several sol-

vent systems is not a sufficient criterion of radiochemical purity for a compound labeled by exposure to tritium gas. After establishing constant specific activity, it is necessary to confirm the radiochemical purity by 1, or preferably several, multistage tests such as thin-layer, paper, or gas chromatography or countercurrent distribution (1-4).

Establishing chemical purity of the final product by elemental and spectral analysis, although of little value in determining the radiochemical purity of a compound labeled by exposure to tritium, is necessary to be certain that the compound will exert its normal biological activity when used in metabolism studies. This is particularly important when the labeled compound is to be administered to humans.

TABLE I.—EXPOSURE OF SULFONYLUREAS TO TRITIUM

Exposure No.	Compd.	Form	Wt., Gm.	Exposure, curie Days
1	I	Salt	1.00	82.5
2	II	Salt	2.00	184
3 ^h	II	Free	1.10	49
4	III	Salt	1.00	82.5
5	IV	Salt	1.25	84
6 ^h	IV	Free	1.10	27.3
7 ⁱ	V	Salt	1.25	42
8	V	Salt	1.25	92
9 ^h	V	Free	1.10	51
10	VI	Salt	2.00	172
11 ^h	VI	Free	1.10	54.5
12 ⁱ	VII	Salt	1.25	42
13 ^h	VII	Free	1.10	49

^a Tolbutamide. ^b Chlorpropramide. ^c Metahexamide. ^d Cycloheptolamide. ^e Tolazamide. See Reference 5. ^f Glypizamide. See Reference 5. ^g See Reference 5. ^h Exposure made by Tracerlab, Inc. ⁱ Exposure made by New England Nuclear Corp.

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TABLE II.—EXPOSURE OF SULFONYLUREAS TO TRITIUM WITH ELECTRICAL DISCHARGE

Exposure No.	Compd. ^a	Form	Wt., Gm.	Tritium Gas, curies	Exposure Time, min.
14	I	Salt	1.00	2.5	20
15	III	Salt	1.00	2.5	20

^a See Table I for structures.

TABLE III.—RESULTS OF EXPOSURE OF SULFONYLUREAS TO TRITIUM

Exposure No.	Compd. ^a	Recrystallizations, No.	Yield, ^b Gm.	Specific Activity, ^b $\mu\text{c./mg.}$	Incorp. of Stably Bound Tritium, ^b $\mu\text{c./curie-Day Exposure}$
1	I	3 ^{c,d}	0.699	14.8	165
2	II	10 ^{e-h}	0.597	16.1	161
3	II	6 ^{e,f}	0.241	1.46	32.8
4	III	3 ^{e,d}	0.618	7.27	82
5	IV	4 ^{e,g}	0.376	10.8	150
6	IV	5 ^{e,g}	0.255	2.76	111
7	V	3 ^{e,g}	0.308	2.68	74
8	V	3 ^{e,i}	0.509	5.3	67
9	V	4 ^{e,g}	0.543	0.64	13.2
10	VI	5 ^{e,i,j}	0.954	12.6	137
11	VI	3 ^{e,i}	0.895	0.62	12.5
12	VII	4 ^{e,i}	0.095	3.36	92
13	VII	4 ^{e,i}	0.259	0.78	17.5

^a See Table I for structures and form exposed. ^b Based on free sulfonylurea. ^c Methanol-water. ^d Ethyl acetate-heptane. ^e Ethanol-water. ^f Benzene. ^g Ethyl acetate. ^h Acetone-water. ⁱ Methyl ethyl ketone. ^j Benzene-heptane.

EXPERIMENTAL AND RESULTS

Preparation of Samples for Exposure to Tritium Gas.—The sodium salts of the sulfonylureas were prepared by adding a molar equivalent of 1 *N* aqueous sodium hydroxide solution to a rapidly stirred suspension of the finely ground compound in water. The resulting solution was lyophilized to obtain the desired sulfonylurea salt as a finely divided solid suitable for exposure to tritium gas. The free sulfonylureas were prepared for exposure by grinding them to a very finely pulverized state in a mortar. Particle-size determinations were not made in either case.

Exposure to Tritium Gas.—The samples were exposed to carrier-free tritium gas under the conditions noted in Table I.¹ When exposures were made in this laboratory, tritium gas was transferred by means of a Toepler pump into an ampul containing the material at a pressure of 10^{-5} to 10^{-6} mm. of mercury. The ampul and its contents under approximately 0.2 Atm. of tritium were stored in the dark at room temperature for an appropriate period of time, usually 2 to 4 weeks. At the end of the exposure period, the waste tritium gas was removed by means of the Toepler pump to leave the crude tritiated material at a pressure of 10^{-5} to 10^{-6} mm. of mercury.

Exposure to Tritium Gas with Electrical Discharge.—The discharge was carried out in a cell similar to the ones described by Dorfman and Wilzbach (6) and Jackson *et al.* (7) at a tritium pressure of approximately 15 mm. using a Tesla coil leak detector as a source of voltage. The sample was suspended in a glass cylinder between the 2 electrodes. Conditions used in the exposures are noted in Table II.

Purification.—Following exposure to tritium gas, each crude sample was precipitated from alkaline solution by addition of a slight excess of acid and

then filtered and washed with water. This procedure was carried out 3 times to remove labile tritium from the sample completely. During one of the precipitations, the alkaline solution was treated with activated charcoal (Darco G-60). Some removal of high specific activity impurities was effected by this treatment. Samples were then recrystallized to constant specific activity using 2-4 solvent systems and 3-10 recrystallizations. The state of radiochemical purity was followed by paper chromatography and by scanning for radioactivity as described below. The number of recrystallizations required, solvent systems used, weight yield, specific activity, and stably bound tritium incorporated for each sample are presented in Tables III and IV.

Determination of Chemical Purity.—Ultraviolet and infrared spectra of each purified sample corresponded to authentic standards. Elemental analyses—carbon, hydrogen, nitrogen, and sulfur (chlorine for those containing this element)—were satisfactory (within $\pm 0.3\%$ absolute of theory) in each case.

Determination of Radiochemical Purity.—Each sample was subjected to paper chromatography in the 1-butanol-piperidine-water (81:2:17 by volume) system on Whatman No. 1 paper and to thin-layer chromatography in the chloroform-formic acid (95:5 by volume) system on silica gel GF. The compound was located in each case by fluorescence quenching under short-wave ultraviolet light. The developed paper chromatograms were scanned for radioactivity by means of a 4- π windowless, paper-strip scanner² having a counting efficiency of approximately 1-2% for tritium. The developed thin-layer chromatograms were first sprayed with a plastic dispersion³ and allowed to dry at room temperature to give the silica gel a paper-like consistency

² Vanguard Instrument Co., LaGrange, Ill.

³ Marketed as Neatan by Brinkmann Instruments, Inc., Great Neck, N. Y.

¹ Certain of the exposures, as indicated in Table I, were carried out by New England Nuclear Corp. or Tracerlab, Inc.

TABLE IV.—RESULTS OF EXPOSURE OF SULFONYLUREAS TO TRITIUM WITH ELECTRICAL DISCHARGE

Exposure No.	Compd. ^a	Recrystallizations, No.	Yield, ^b Gm.	Specific Activity, ^b $\mu\text{c./mg.}$
14	I	5 ^{c,d}	0.514	3.67
15	II	5 ^{c,d}	0.479	3.57

^a See Table I for structures. ^b Based on free sulfonylurea. ^c Methanol-water. ^d Ethyl acetate-heptane.

and thus facilitate its handling. The silica gel film was then cut into sequential 0.5-cm. sections (usually 30) which were transferred to individual counting vials. One-half milliliter of dimethylformamide was added to each vial to dissolve the plastic dispersion,³ releasing the silica gel, and the series of vials was counted as described below. Each purified sample showed a single radioactive zone in each chromatographic system corresponding to the zone of fluorescence quenching which in turn corresponded to the migration of the authentic sulfonylurea.

Radioactivity Measurements.—All counting was performed with a Tri-carb⁴ model 314X or 314EX-2A liquid scintillation spectrometer at -8° under conditions suitable for measuring tritium. Suitable aliquots of samples were dissolved in 15 ml. of diotol scintillator [toluene-dioxane-methanol (350:350:210 by volume) containing 73 Gm. of naphthalene, 4.6 Gm. of 2,5-diphenyloxazole, and 0.080 Gm. of 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter]. The absolute counting efficiency (usually 12 to 16%) for each sample was determined by addition of an internal standard of tritium-labeled toluene and results then expressed as microcuries.

DISCUSSION

As shown in Table III, the sulfonylureas were more satisfactorily labeled with tritium by exposure of their sodium salts, rather than the free compounds, to tritium gas. The sodium salts incorporated 5 times as much stably bound tritium on the average as did the free sulfonylureas. It is possible that in the case of the free sulfonylureas the tritium tends to localize at the labile $-\text{NH}-$ position, diluting the tritium gas with hydrogen and thereby decreasing the rate of exchange of tritium with the more stable hydrogens. Blocking the labile position by formation of the sodium salt may thus allow tritium to exchange with stable hydrogens at a more favorable rate. Other explanations, such as more self-destruction of the desired labeled species when a large quantity of tritium is present in a labile position or a larger particle size or less porous surface in the cases of the free sulfonylureas, although speculative, are also possible.

Incorporation of tritium into stable positions of the sulfonylurea sodium salts was remarkably uni-

form for the series of 7 compounds, ranging from 67 to 165 $\mu\text{c.}$ per curie-day exposure. In the single case where 2 samples of the same sulfonylurea sodium salt (compound V, Table III) were tritiated, the incorporations were in good agreement, 67 and 74 $\mu\text{c.}$ per curie-day exposure, even though substantially different exposures were made in different laboratories.

Incorporation of stably bound tritium into the free sulfonylureas did not appear to be nearly so uniform, ranging from 12.5 to 111 $\mu\text{c.}$ per curie-day exposure. However, except for the high value of 111 $\mu\text{c.}$ per curie-day exposure (compound IV, Table III), the range of incorporations (12.5 to 32.8 $\mu\text{c.}$ per curie-day exposure) was no greater than that of the sodium salts.

Compounds I and III (structures in Table I) were also tritiated by exposure to tritium gas in the presence of an electrical discharge as summarized in Tables II and IV. These tritiations were successful although the specific activities attained were not as high as those obtained by the conventional exposure method. Increasing the discharge time might have increased incorporation of stably bound tritium, but it probably would also have increased degradation (6). Even with a 20-min. discharge, more degradation appeared to take place than was the case where conventional exposure conditions were used. It is interesting to note that the specific activities of the 2 sulfonylureas prepared by the discharge method were essentially the same, whereas the specific activity of I was twice that of III when the conventional exposure method was used. These results may be due to a difference in mechanisms or a relative difference in the contributions of several mechanisms leading to incorporation of tritium by the 2 methods as discussed by Ache *et al.* (8).

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⁴ Packard Instrument Co., Inc., Downers Grove, Ill.