TRITIUM LABELING OF SIMPLE 7-MEMBERED RING COMPOUNDS

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SUMMARY

Seven-membered ring compounds, from cylcoheptane to complex ring structures containing heteroatoms, substituents and fused phenyl rings, were labeled with tritium, using activated and adsorbed tritium. The 7-membered ring structures are generally stable towards reactions with tritium, which allows compounds like 1-benzosuberone, 1-aza-2-methoxy-1-cycloheptene, iminostilbene and clozapine to be labeled to reasonably high specific activities. The best method varies greatly from compound to compound. By optimizing the labeling conditions and use of efficient support exceptionally good results can be obtained. The Pd-on-alumina support gives consistently higher specific activity and less radioimpurity than other supports. Even molecules containing carbon-halogen bond and hydrogen bound to nitrogen can usually be labeled with tritium at stable positions and without dehalogenation.

Key Words: Tritium labeling, microwave discharge, catalyst support, 7-membered rings, gas-liquid radiochromatography.

INTRODUCTION

Biologically active substances and neuroleptic drugs such as benzazepines, benzodiazepines and many tricyclic antidepressants have in common a 7-membered ring in their structure. These 7-membered ring compounds can be labeled by multi-step radiosynthesis or catalytic isotopic exchange with tritium. Buchman et al. reported the labeling of fourteen tricyclic antidepressants containing a 7-membered central ring [1]. Compounds with dihydrobenzoazepine skeleton were labeled by isotopic exchange at benzylic hydrogen but those with higher specific activities were obtained by tritiodebromination of precursors in alkaline medium in the presence of PdO with tritium gas. Zolyomi et al. synthesized labeled amitriptyline from labeled dibenzosuberone [2]. Brundish labeled carbamazepine by selective catalytic reduction of the brominated precursor with tritium gas [3]. The antipsychotic drug, SCH 23390, a derivative of

0362–4803/90/050543–12$06.00

Received August 30, 1989
Revised November 2, 1989

benzazepine, has been labeled by reductive aryl debromination of a brominated precursor with tritium [4]. Clozapine, a dibenzodiazepine structure with N-methyl piperazine substituent, has been labeled to specific activity of 0.56 mCi/mmol by a difficult synthesis [5]. Recently, de Paulis and coworkers synthesized [^{3}H]clozapine with a specific activity of 9.9 Ci/mmol from precursors containing tritium label in the N-methylpiperazine ring [6]. Liebman has reviewed the isotopic labeling of benzodiazepines [7]. Benzodiazepines possessing a methyl group attached to ring nitrogen are relatively easy to label but the methyl group is often rapidly metabolized in vivo; furthermore not all benzodiazepines contain such group.

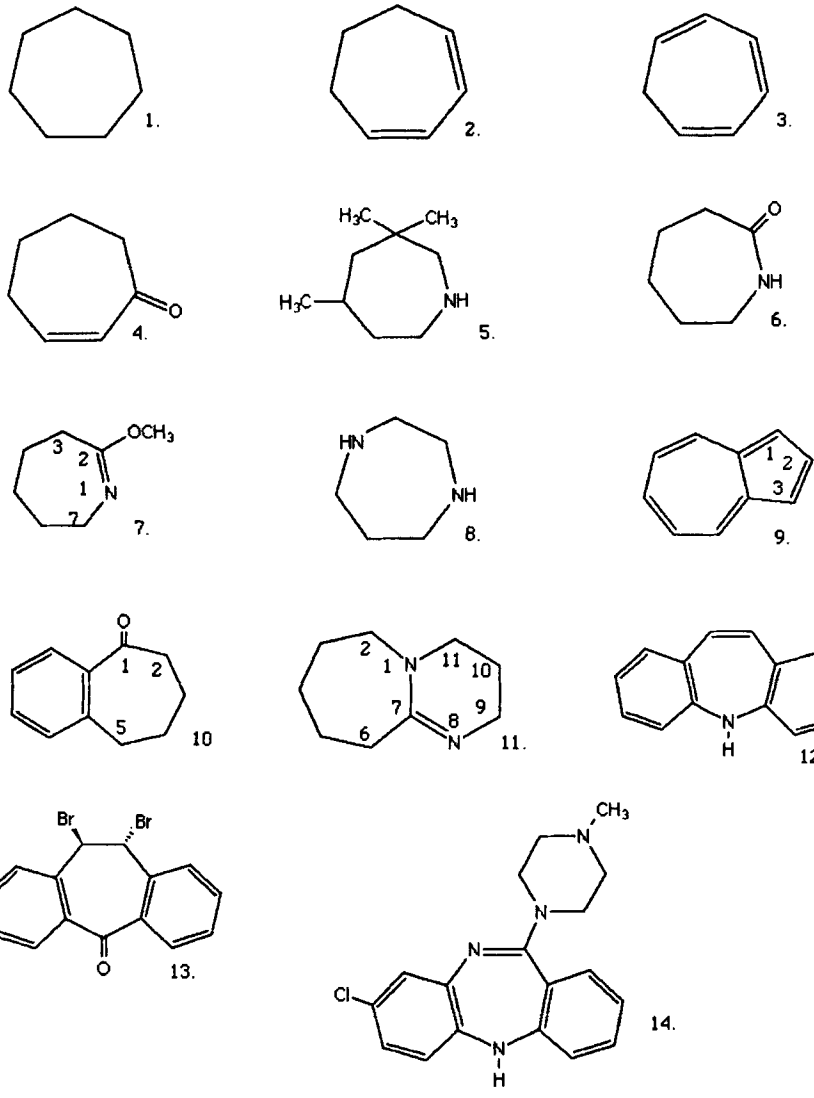
Benzodiazepines of extremely high specific activity required for receptor binding studies are labeled by synthesis [7]. For in vitro and metabolic studies, labeled materials of such high specific activity are not usually required. Labeling using adsorbed tritium or activated tritium is direct and fast, requiring no synthesis of precursors, and can yield labeled compounds with reasonably high specific activity [8,9]. We have applied this method to the labeling of 14 compounds containing a 7-membered central ring. Our observations on (i) the stability of the 7-membered ring towards reactions with tritium, (ii) the effects of substituents and heteroatoms on isotopic exchange, (iii) the effects of catalyst and labeling conditions on yield, and (iv) the stability of the tritium label in the molecule, are reported here.

RESULTS AND DISCUSSION

The structures of the 7-membered ring compounds that we have studied are given in Figure 1. These structures increase in complexity from the simple 7-membered ring cycloheptane to the complicate benzodiazepine systems which contain double bonds and nitrogen atom(s) in the central ring with peripheral substituents or fused phenyl ring(s). Seven of the 14 compounds studied are liquid at room temperature and the others are solid. These compounds were labeled using the activated tritium (AcT), adsorbed tritium (AdT) and high-temperature-tritium-ion (HTI) methods [10,11]. The labeled compounds were purified, radioassayed and analyzed according to the procedures described under METHODS. Table 1 lists the yields and specific activities of the above compounds labeled with tritium using different methods. Table 2 gives the by-products and radioimpurities formed in the same reactions as listed in Table 1.

As shown in Table 1, the specific activities of the labeled products vary over a wide range, from a few millicuries to curies per millimole. The simplest compounds cycloheptane (**1**), 1,3-cycloheptadiene (**2**), 1,3,5-cycloheptatriene (**3**) and 2-cyclohepten-1-one (**4**) can be labeled with activated tritium and adsorbed tritium to medium specific activities, with formation of by-products. The number in parenthesis corresponds to the same in Table 1. Saturated alicylic hydrocarbons are inert and difficult to label, which accounts for their medium specific activity, while the keto compound can be labeled to a higher specific activity. Activated tritium has a kinetic energy of less than 4 eV, insufficient to break the carbon-carbon bond on impact but can add to the double bond [9]. The diene and triene can yield by-products, such as [^{3}H]cycloheptane (**1**), [^{3}H]cyloheptene, etc., which are formed from the parent by tritium addition in stages and are in no-carrier-added state with a specific activity approaching 29 Ci/mMol. Cycloheptane (**1**) can yield [^{3}H]methylcyclohexane and [^{3}H]heptane as by-products by rearrangement and ring opening catalyzed on the surface of the supported metal catalyst. These are minor reactions and unique only

Figure 1. Structures of the labeled compounds



1. Cycloheptane
2. 1,3-Cycloheptadiene
3. 1,3,5-Cycloheptatriene
4. 2-Cyclohepten-1-one
5. (±)3,3,5-Trimethylhexahydroazepine
6. 2-Oxohexamethyleneimine (caprolactam)
7. 1-Aza-2-methoxy-1-cycloheptene
8. 1,4-Diazacycloheptane (homopiperazine)
9. Azulene
10. 1-Benzosuberone
11. 1,8-Diazabicyclo-[5,4,0]undec-7-ene
12. 5H-Dibenzo[b,f]azepine (iminostilbene)
13. *trans*-10,11-Dibromodibenzosuberone
14. 8-Chloro-11-(4-methyl-1-piperazinyl)5H-dibenzo[b,e][1,4]diazepine (clozepine)

Table 1. Yields and specific activities of 7-membered ring compounds labeled by different methods.

No.	Compound	Method[a]	Yield crude (mCi)	Yield purified (mCi)	Spec. Act. mCi/mMol	Remarks
1.	Cycloheptane	A	51	10.5	31.8	b
2.	1,3-Cycloheptadiene	B	127	8.6	8.9	b
3.	1,3,5-Cyclo - heptatriene	A	24	6.8	17	b
4.	2-Cyclohepten-1-one	C	272	134	157	c
5.	(±)3,3,5-Trimethyl-hexahydroazepine	A	72	2.4	16	isomers present
6.	Caprolactam	C	369	166	107	
7.	1-Aza-2-methoxy-1-cycloheptene	D	322	299	428	~14% d
8.	Homopiperazine	C	219	31	48	
9.	Azulene	C	54	48	185	c
10.	1-Benzosuberone	E	193	172	1833	c
11.	1,8-Diazobicyclo-[5,4,0]undec-7-ene	C	175	101	151	~6.5% d
12.	Iminostilbene	C	491	117	238	~1.8% d
13.	*trans*-10,11-Dibromo-dibenzosuberone	C	52	17	43	
14.	Clozapine	F	23	2	562	

[a] A = labeled with activated tritium with substrate dispersed on silica alumina #980-25; B = labeled with adsorbed tritium on 1% Ni on 980-25; C = labeled using the high temperature-tritium ion method with 1% Pd on alumina catalyst, A1-3945; D = labeled using the high temperature-tritium ion method on 4.6% Pd on granulated charcoal not activated by heating with hydrogen. E = labeled with activated tritium with substrate dispersed on 1% Pd on A1-3945; F = labeled with activated tritium with substrate dispersed on Millipore filter.

[b] Products of ring saturation, ring rearrangement and degradation were found.

[c] Single experiment

[d] Radioimpurities

Table 2. Yield of labeled parents and by-products formed in the labeling of some 7-membered ring compounds. The identity of the by-products is based on their retention indexes on apolar and polar columns.

No.	Labeled parent and by-products	Retention indexes DB-1	CB-20M	Yield %
1.	[^{3}H]Cycloheptane	796	883	85
	[^{3}H]heptane		690	8
	[^{3}H]methylcyclohexane		766	7
2	[^{3}H]1,3-Cyclohetadiene	811	1051	7
	[^{3}H]cycloheptane		880	62
	[^{3}H]cycloheptene		921	31
3.	[^{3}H]1,3,5-Cycloheptatriene	785	1084	34
	[^{3}H]cycloheptane		879	58
	[^{3}H]cyclohepte		923	5
	[^{3}H]cycloheptadiene	811	1050	2
4.	[^{3}H]2-Cyclohepten-1-one		1650	79
	[^{3}H]impurity		1483	1
	[^{3}H]cycloheptanone		1558	19
	[^{3}H]impurity		2109	1
5.	[^{3}H]Homopiperazine	936		89
	[^{3}H]impurity	1127		11
6.	[^{3}H]Caprolactam	1230	2295	100
7.	[^{3}H]Trimethylhexa-hydroazepine	1034	1320	47
			1265	16
	[^{3}H]impurity		1418	21
8.	[^{3}H]Azulene	1280	2081	100
9.	[^{3}H]1-Benzosuberone	1415	2288	96
	[^{3}H]impurity	1379	2027	0.3
		1390		2
		1463		2
10.	[^{3}H]1-Azamethoxycycloheptane	976	1307	88
	[^{3}H]impurity	1222		12
11.	[^{3}H]1,8-Diazabiocyclo-undecane	1375		100
12.	[^{3}H]Iminostilbene	1920		98
	[^{3}H]dihydroimino-stilbene	1827		2
13.	[^{3}H]Dibromodibenzo-suberone	2014		77
	[^{3}H]impurity	2241		10
	[^{3}H]impurity	2274		13
14.	[^{3}H]Clozapine	2816		100

to cycloheptane. Had they been otherwise, more of these products would have been detected in the labeling of all other 7-membered ring compounds. In the labeling of steroids we found that radioimpurities not related to the parent compound are formed from extraneous sources [9]. This is also the case with 7-membered ring compounds that traces of impurities contained in the commercially supplied compounds are usually more efficiently labeled to high activities than the compound itself.

Compounds such as trimethylhexahydroazepine (**5**), caprolactam (**6**), homopiperazine (**8**) and iminostilbene (**12**) contain hydrogen bound to nitrogen. Activated tritium can undergo isotopic exchange readily with hydrogen attached to ring nitrogen and also with hydrogen at the α-position to the keto group. Labels in these positions are labile and can back-exchange. Tritium nmr spectroscopy shows that trimethylhexahydroazepine (**5**) dispersed on 1%Ni on silica-alumina (980-25) and labeled with activated tritium has the label entirely bound to nitrogen. The HTI method can label caprolactam (**6**), homopiperazine (**8**) and iminostilbene (**12**) on Pd-on-alumina as support at stable positions. The labeled products can be rigorously purified and lyophilized to achieve constant specific activity. At the room temperature the isotopic exchange with tritium is limited to labile sites; at high temperatures the exchange also occurs at stable positions.

Both the AdT and the HTI methods can label azulene (**9**), diazabicycloundecene (**11**), benzosuberone (**10**) and azamethoxycycloheptene (**7**). The silica-alumina is highly acidic [10] and will bind a small amount of the above substrates to the matrix tightly to make elution difficult. In fact, no labeled substrates were recovered from the method of activated tritium. Changing support may avoid the irreversible binding. For example, clozapine (**14**) dispersed on Millipore filter (HVLP) and l-benzosuberone (**10**) on 1% Pd-on-alumina were labeled by activated tritium to specific activity of 562 and 1,888 mCi/mmol, respectively. Other supported metal catalysts were also found effective but yielded products of lower specific activity.

Dibromodibenzosuberone (**13**) when labeled by the HTI method yielded [^{3}H]dibromo-dibenzosuberone. During the reaction, debromination was anticipated but no [^{3}H]benzosuberone (**10**) was detected in the final product. According to our earlier observation, the adsorbed tritium causes no dehalogenation of halogenated compounds [8]. Clozapine (**14**) when labeled using the AdT and HTI methods gave poor yields, but [^{3}H]clozapine of high specific activity was obtained using the AcT method when the substrate was dispersed on Millipore (HVLP) membrane. These data clearly demonstrate that labeling is dependent on method and conditions of labeling.

Table 3 lists the radiochemical yields and specific activities of [^{3}H]l-benzosuberone (**10**) and [^{3}H]l-aza-2-methoxy-1-cycloheptene (**7**) obtained using different labeling methods and conditions. These data show very interesting differences regarding methods and substrates. The HTI and the AdT methods use larger amounts of samples and give higher yields and fewer radioimpurities than the AcT method. l-Benzosuberone (**10**) exhibits less tight binding to the matrix than l-aza-2-methoxy-1-cycloheptene (**7**). Both metal and support play important roles in the exchange. Among all the supports, 1% Pd(A1-3945) gave consistently good results. Activated tritium can label 1-benzosuberone (**10**) on 1% Pd (Al-3945) support to very high specific activity with very little radioimpurities but cause partial saturation of the fused phenyl ring on 1% Ni(silica gel) support. The best method for labeling l-aza-2-methoxy-1-cycloheptene (**7**) is by the HTI method on 4.6% Pd on charcoal pellets, although other supported metal catalysts are also effective.

Table 3. Specific activities, yields and radioimpurities found in the tritiation of 1-benzosuberone and 1-aza-2-methoxy-1-cycloheptene by different methods

Methods of labeling	Yield mCi	Specific activity mCi/mmol	Percent of impurities
1-Benzosuberone			
AcT/(980-25)	0.22	2.20	a
AcT/1% Ni(980-25)	0.10	1.01	a
AcT/0.5% Ru(980-25)	0.32	3.22	a
AcT/V(980-25)	0.19	1.91	a
AcT/1% Pd(A1-3945)	172	1833	3
AcT/1% Ni(silica-gel)	10.3	110	73
AdT/1% Ni(980-25)	72	108	ND[b]
AdT/1% Ni(A1-3945)	154	231	1.7
HTI/1% Pd(A1-3945)	227	340	2.0
HTI/5% Pd(A1-3945)	181	272	ND
HTI/4.6% Pd(carbon) (activated)	04	1.55	ND
HTI/4.6% Pd(carbon) (non-activated)	2.96	1.98	ND
HTI/1% Ni(silica-gel)	7.40	11.0	5.0
1-Aza-2-methoxy-1-cycloheptene			
AcT/980-25	no labeled product formed		
AcT/1% Ni(980-25)	no labeled product formed		
AcT/0.5% Ru(980-25)	no labeled product formed		
AcT/V(980-25)	no labeled product formed		
AcT/1% Pd(A1-3945)	65.6	7.73	9.4
AcT/1% Ni(silica gel)	18.3	2.16	ND
AdT/1% Ni(980-25)	288	175	9.0
AdT/1% Ni(A1-3945)	12.1	17.4	14.9
HTI/1% Pd(A1-3945)	189	132	55.8
HTI/5% Pd(A1-3945)	20.5	15.0	15.0
HTI/4.6% Pd(carbon) (activated)	2.14	1.49	
HTI/4.6% Pd(carbon) (unactivated)	428	299	14.0
HTI/1% Ni(silica gel)	33.3	16.9	12.0

[a]Partial saturation of phenyl ring
[b]ND = not detected.

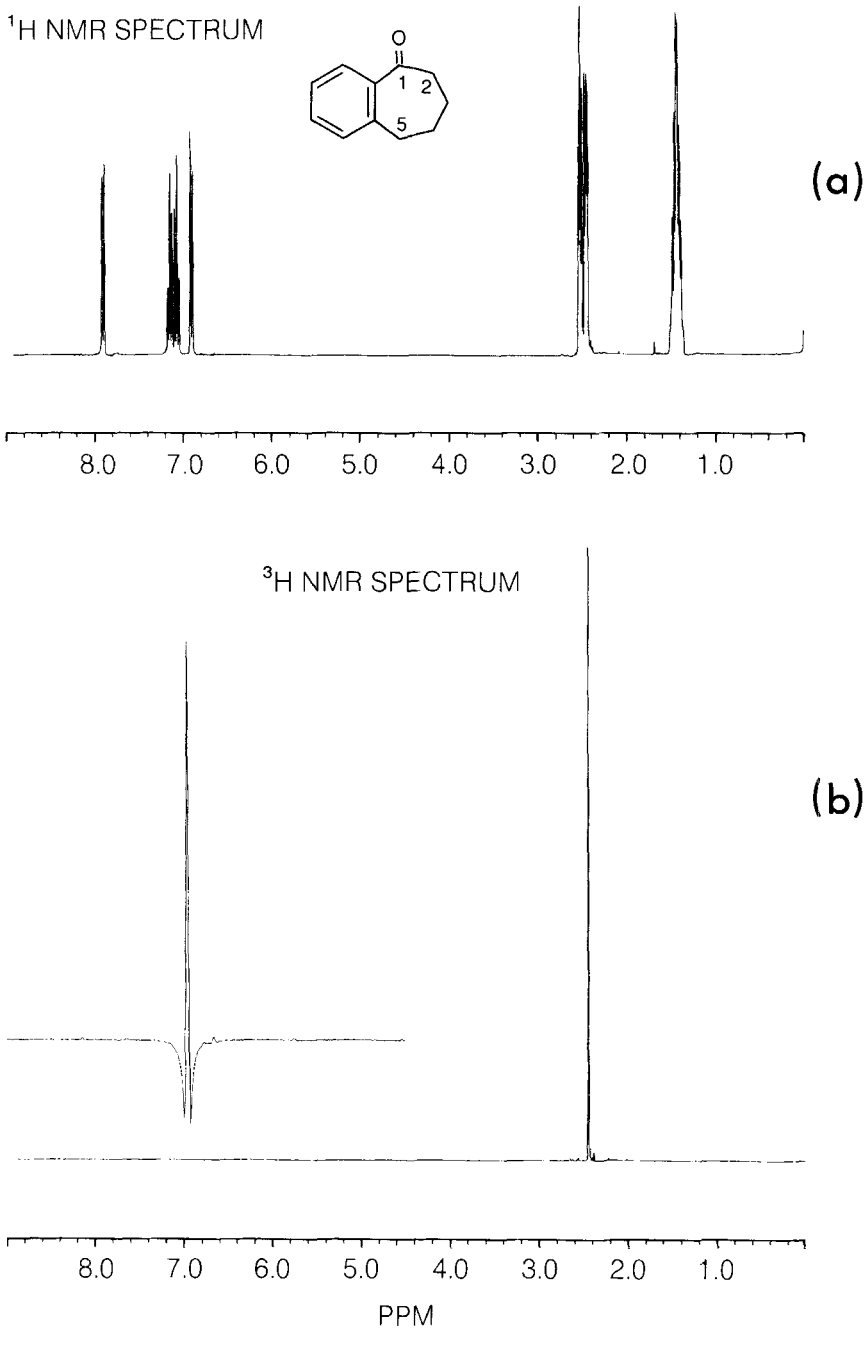


Figure 2. Tritiated 1-benzosuberone (**10**): (a) Proton NMR spectrum and (b) "proton decoupled" ^{3}H NMR spectrum.

The usefulness of compounds labeled by this method is dependent upon the distribution of tritium label in stable positions in the molecule that will not back-exchange. Measurement by ^3H nmr spectroscopy can reveal such distribution [12]. A few examples are given here. The "proton decoupled" ^3H nmr spectrum and the proton nmr spectrum of [^3H]benzosuberone (**10**) in deuterated benzene are given in Figure 2, showing the tritium label to be in positions 2 and 5. These two resonances are not well resolved because of their very similar chemical shifts. Tritium in position 2 is α to the keto group and labile; after purification, the labeled compound should contain only stable tritium in position 5 (s, δ 2.44, H-5). The spectrum shown in Fig. 2b supports this assignment. Other measurements in deuterated benzene show that [^3H]azulene (**9**) has the tritium label in positions 1 and 3 in the five-membered ring (s, δ 7.26, H-l, H-3), and that [^3H]1,8-diazabicyclo-[5,4,0]undec-7-ene (**11**) has the label entirely at position 6 (s, δ 2.39, H-6). [^3H]1-Aza-2-methoxy-1-cycloheptene (**7**) measured in deuterated acetone has the label either at position 3 (s, δ 2.35) possibly at position 7, since these two resonances cannot readily be unequivocally resolved. The fact that activated and adsorbed tritium can label these compounds in a specific position or specific positions is contrary to the long held perception that radiation induced methods are only capable of yielding randomly labeled products. Tritium nmr spectroscopy can determine the tritium distribution in labeled molecules without chemical manipulation [12]. This study shows that the 7-membered ring compounds can be labeled in chemically stable specific positions by isotopic exchange with tritium and the labeled products are suitable for use in radiotracer studies.

CONCLUSION

Labeling the 7-membered ring compounds using the methods of activated tritium, adsorbed tritium at room temperature and adsorbed tritium at high temperature, is direct, fast and can generally yield labeled products of medium to high specific activity. The ring is stable towards reactions with activated tritium. Heterocyclic rings undergo tritium exchange more readily than homocyclic rings. The exchange is specific and not random. Both catalytic supports and the labeling conditions can influence the labeled product with respect to yield, specific activity, formation of by-products and radioimpurities. Radioimpurities are likely formed from extraneous sources. In the HTI method tritium exchange occurs at chemically stable positions, even though the molecule may contain labile sites. The best method for labeling appears to vary greatly from compound to compound. The use of tritium nmr spectroscopy to determine the distribution of tritium label is essential.

MATERIALS AND METHODS

All the compounds except clozapine were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin) and were used without further purification. Clozapine (14) was kindly supplied by Sandoz (Basle, Switzerland).

1. Activated tritium (AcT) method [10]

Different catalyst supports, untreated or impregnated with 0.5% to 5.0% of various metals

(Ru, V. Ni, Pd) were used as supports for dispersing the compound to be labeled (the substrate). The support materials used were silica-alumina #980-25 in 3mm x 3mm pellets (Davison Chemical Co., Baltimore, MD. USA), alumina catalyst A1-3945 in 0.63mm dia. extruded sections(Harshaw Chemical Co., Elyria, OH, USA), silica gel and activated carbon pellets (6-8 mesh, Alfa Division, Danvers, MA). Plain Millipore filter (HVLP) was also used as support. Preparation of these supports followed the procedure described earlier [13]. The following supports were prepared: silica-alumina #980-25 containing 0.5% Ru, 1% Ni or 1%V; A1-3945 1% Ni, 1% Pd or 5.0% Pd; silica 1.0% Ni and carbon 4.6% Pd either activated or non-activated. The metal catalysts were activated by heating to 600°-700°C in hydrogen. Untreated silica-alumina was also used for support.

Liquid samples (5-10 μl) were pipetted directly onto each pellet. Solid samples were dissolved in a suitable solvent, and the solution was pipetted onto the pellets. The impregnated pellets were thoroughly dried in vacuo prior to labeling.

Samples were placed in a tritiation apparatus similar to the one described earlier [14]. The apparatus containing the sample was evacuated to less than 3 millitorr before admitting tritium gas which was freshly generated on a daily basis by heating uranium tritide to 350-400°C. The tritium pressure used varied from 2.7 to 5.2 torr. A microwave power generator (500-W, MicroNow, Chicago, Il) was used to supply power, via an Evenson cavity, to maintain the plasma. The power varied from 40 to 120 W with a current of 40-100 mA. The sample was placed downstream from the discharge for a duration of 5 min. Depending of the size of the pellet, 5 to 10 pellets were exposed at one time. After the exposure, the spent tritium was pumped to a waste storage tank for disposal. The apparatus was flushed with He and opened to atmosphere for sample retrieval.

2. Adsorbed tritium (AdT) method [10]

The supported metal pellets were exposed to a tritium plasma generated with 30-50 W power at 50 mA for 20 minutes. The tritium pressure was varied from 3.9 to 7.0 torr. After exposure and removal of tritium gas, the "tritium charged" pellets were dropped into approximately 100 μl of the liquid and mixed to let the exchange occur.

3. High temperature-tritium ion (HTI) method [11]

The above AdT method was adapted for labeling solid samples by heating the mixture containing the tritium-charged pellets and the substrate (20-200 mg) briefly above its melting point.

4. Purification and analysis

Labile tritium in the products was removed by equilibration with hydroxylic solvents, lyophilization or extraction. The labeled products were radioassayed by liquid scintillation counting and analyzed by gas-liquid radiochromatography (GLRC) on a 15-m (0.53mm dia.) fused silica capillary DB-1 column (J&W Scientific) and a 2.5-m (3 mm dia.) stainless steel column packed with Carbowax 20M (CB-20 M, 10% on Supelcoport, Supelco Inc.), using a Hewlett-Packard 5580A or 5890 gas chromatograph equipped with thermal conductivity detector. The radioactivity of the column effluent, after mixing with n-propane, was measured with a proportional counter at 250°C. The radioactive peaks were integrated with a Tracor-Northern Model 7200 multichannel analyzer, operating in the MCS mode and the output was recorded by a Teletype Corporation

Model 43 keyboard printer. A standard mixture of n-alkanes from 5 to 32 carbon atoms was injected together with the sample for retention index calculation. The unknown radioactive peaks were tentatively identified by their retention indexes, using the structure retention index relationship (SRIR) developed earlier [15]. By this method it is possible to differentiate by-products from radioimpurities; the latter are formed from extraneous sources (Cf. Table 3).

Labeled compounds that cannot be purified by GLRC were purified by isocratic reversed-phase HPLC on μBondpak C18 columns (Waters) using methanol:water (70:30) as mobile phase. Separation by HPLC was carried out in a Beckman HPLC system (Model 421A Controller and two Model 110 pumps) and a Kratos Spectroflow 757 absorbance detector. Samples were separated by isocratic elution at a flowrate of 1.0 ml/min of the the mobile phase. Both iminostilbene (**12**) and dibromodibenzosuberone (**13**) with retention volumes of 6.7 ml and 5.0 ml, respectively, were readily analyzed.

5. Tritium nmr spectroscopy

Samples were made to a volume of 200 μl in teflon tubes (Wilmad, #6005), which were placed inside 5mm glass nmr tubes having screw caps (Wilmad, 507-TR-8"). The NMR spectroscopy was carried out on an IBM Instruments Inc. AF-300 spectrometer (^{3}H at 320 MHz, ^{1}H at 300 Mhz), using ^{3}H/^{1}H 5mm dual probe. Referencing of chemical shifts was achieved by generation of a ghost ^{3}H TMS signal in ^{1}H nmr spectrum [12].

ACKNOWLEDGEMENTS

We wish to acknowledge research support from the National Cancer Institute, DHHS (PHS grant number CA 33537), Cultural Foundation of Finland and the National Tritium Labeling Facility (supported by NIH grant, RR 01237). We thank P.G. Williams for the ^{3}H NMR spectra and H. Morimoto for technical assistance in tritium handling.

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