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TRITIATION OF ORGANIC COMPOUNDS BY POLYMER-SUPPORTED ACID CATALYSTS

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SUMMARY

A range of organic compounds have been tritiated (and in some cases deuteriated) via hydrogen isotope exchange reactions using polymer supported acid catalysts. The extent and regiospecificity of labeling were investigated as a function of substrate and catalyst type. Although all the catalysts were found to be active in the tritiation of both aromatic and heterocyclic compounds, there was clear evidence that the extent of hydrogen isotope exchange was influenced by the nature of the polymer support matrix .

Keywords; Polymer supported acid catalyst, hydrogen isotope exchange, tritiation, ion exchange resins, Nafion

INTRODUCTION

Tritium labelled compounds are finding increasing applications in the life sciences[1] partly because of the high specific activities attainable and the relative ease with which the label can be incorporated, two very favourable considerations when a comparison with ^{14}C is made. Furthermore, the development of ^{3}H nmr spectroscopy[2] now provides the analytical reassurance so necessary when employing tritium labelled compounds.

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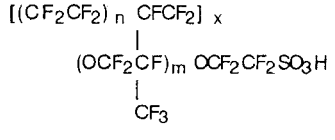
Since the 1960's a range of rapid, one-step catalytic hydrogen isotope exchange procedures have been developed[3] in order to tritiate organic compounds. The catalyst can be in the form of an acid, base or metal but not all of them are equally attractive. Thus some of the highly reactive Lewis acids, such as the organoaluminium halides[4], are pyrophoric whilst fluorinated acids such as heptafluorobutyric acid are corrosive and unpleasant[5]; reagents such as the boron trifluoride-phosphoric acid complex[6] developed by Yavorsky et al attack normal glassware.

In a slightly shorter time interval, a great deal of progress has been made in developing a whole range of polymer supported reagents and catalysts[7]. Rather surprisingly there are relatively few examples[8,9] where polymer supported catalysts have been used to label compounds. In cases where time is important, e.g. the synthesis of ^{11}C butylamine[10] the advantages are obvious and even in hydrogen isotope exchange reactions the ease with which the product can be isolated is a considerable improvement on the customary solvent extraction procedure.

In the present paper we explore the potential for using ion exchange resins, in the acid form, for tritiating a range of model compounds. The work is then extended to a number of more complex substrates using Nafion (A), a much stronger acid, as catalyst, before finally using the deuteriation of 1,4-dimethoxybenzene to study the effect of the polymer support matrix on the exchange reaction. The physical properties of the catalysts are summarised in Table 1.



A

Table 1. Physical properties of the polymer supported acid catalysts.

Catalyst	Support	Structure	Acid Type	Max. Temp. (°C)
Dowex-50W-X8	PS-DVB[a]	gel	SO_3H-strong	150
Amberlyst 15	PS-DVB	macroreticular	SO_3H-strong	120
Amberlyst 1010	PS-DVB	macroreticular	SO_3H-strong	120
Nafion	PAE[b]	cross-linked linear	SO_3H-superacid	200

[a]PS-DVB : Polystyrene-divinylbenzene

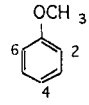
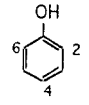
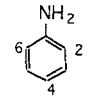
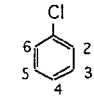
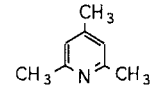
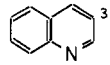
[b]PAE : Perfluorinated aliphatic ether

RESULTS AND DISCUSSION

The labeling conditions together with the tritium incorporation, distribution and ^3H nmr chemical shift data for a number of aromatic and heterocyclic model compounds are given in Table 2. In the case of the aromatic compounds exchange is favoured by the presence of strongly electron releasing substituents ($-NH_2$, -OMe, -OH) with the ortho-para orientation being characteristic of an electrophilic substitution mechanism. Strongly deactivated compounds such as nitrobenzene (not given) could not be labelled using the Dowex type resin.

The results for 2,4,6-trimethylpyridine (V) showed (Fig.1) that both methyl groups had been tritiated. This pattern of regiospecificity is similar to that obtained in dilute solutions of sulphuric acid[11] and as the regiospecificity is strongly dependent on the acidity, gives a measure of the acidity of these resins. This argument is supported by the fact that quinoline (VI) was found to label specifically in the 3 position when using both the Dowex and Amberlyst resins. Katritzky et al[12] found that exchange of the 2 and 3 protons of quinoline was only important over the acidity range pH 0.5 to Ho - 3 at 245°C and that at higher acidities exchange into the C-5, - 6 and - 8 positions was

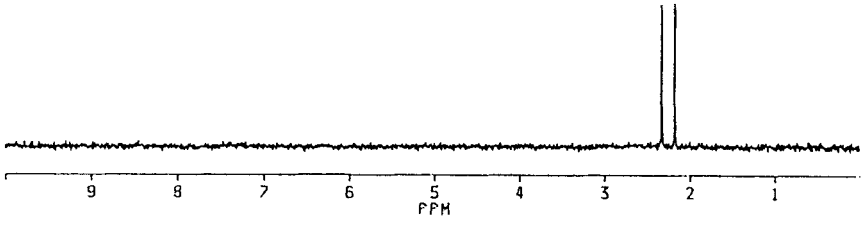
Table 2. Tritiation of Aromatic and Heterocyclic Model Compounds by Dowex 50W-X8[a]

Compound	Structure	T(°C)/h	Total Incorporation (mCi)	Specific Activity (mCi/mmol)	^{3}H Chemical Shift[b] (ppm)	Position	%
I[c]		80/20	25.3	9.9	6.94[d] 6.69	4 2,6	40 60
II		80/16	14.7	7.7	6.80	2,6 +4	overlapping
III		80/60	20.6	6.4	6.66 6.59	2,6 4	66 34
IV		120/60	6.3	2.2	7.43 7.39 7.33	2,6 3,5 4	44 7 49
V		120/36	5.0	2.2	2.34 2.16	CH_3 2,6 CH_3 4	52 48
VI		120/60 120/100[e]	1.6 2.1	0.6 0.8	7.48 7.47	3 3	100 100

[a]Reaction mixture: substrate (0.3cm^3), resin (70mg) and tritiated water of specific activity 5Ci cm^{-3}, (10^{-2}cm^3) Activated compounds 80°C run, less activated compounds 120°C run [b]Chemical shifts in d_6-DMSO unless otherwise stated [c]Catalyst 275mg [d]Chemical shift in $CDCl_3$ [e]Amberlyst 15

favoured. In our studies[13], heating quinoline with tritiated water alone gave a product specifically tritiated in the C-2 position.

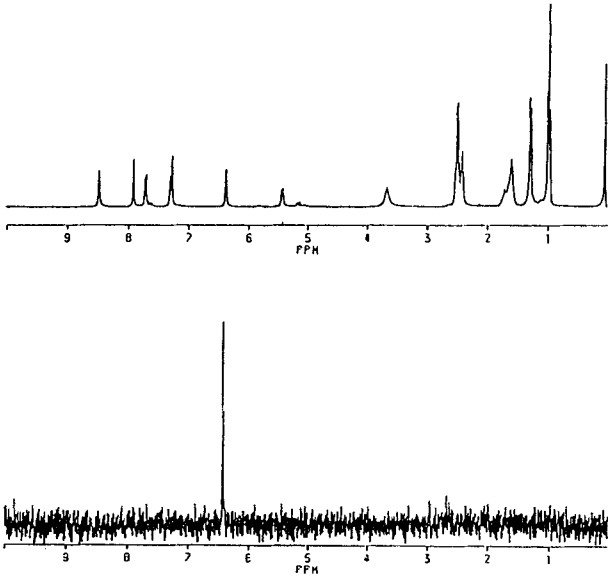
Fig.1 ^{3}H Nmr (proton decoupled) of tritiated 2,4,6-trimethylpyridine



The labeling conditions together with the tritium incorporation, distribution and ^3H nmr chemical shift data for four heterocyclic compounds of biological interest are given in Table 3. For these investigations it was necessary to use the strongest of the four polymer- supported acid catalysts, namely Nafion. Pyridoxine (VII) which is represented in the vitamin B_6 framework, was chosen as a suitable candidate for study because of its structural resemblance to 2,4,6-trimethylpyridine. Good incorporation was achieved with one of the hydroxymethyl groups being labelled as well as the methyl group.

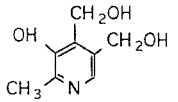
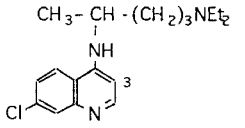
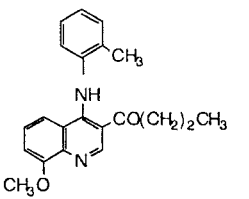
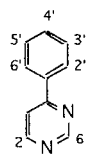
The anti-malarial agent chloroquine (VIII) was tritiated as the free base and although there have been reports[14] of unsuccessful tritiations using both $AlCl_3$ and CF_3CO_2H good tritium incorporation was achieved. The ^1H and ^3H nmr (^1H decoupled) spectra are shown in Fig.2. Specific incorporation into the C-3 position of the quinoline ring system was achieved, consistent with the previous findings for the parent molecule.

Fig.2 ^1H and ^3H Nmr spectra of 3 - ^3H-chloroquine



The substituted quinoline (IX, SK &F 96067) offered several potential sites for exchange but it was the methylene function situated α to the carbonyl group that was specifically tritiated, which may or may not be satisfactory, depending on the well known lability of tritium in that position and the application in mind.

Table 3 Tritiation of Compounds of Biological Interest using Nafion[a]

Compound	Structure	Weight S/C(mg)[b]	T (°C)/h	Total Incorporation (mCi)	Specific Activity (mCi/mmol)	^{3}H Chemical Shift[c] (ppm)	Position	%
VII[d]		40/50	90/8	5.5	1.2	4.77 2.35	$-CH_2O$ CH_3	21 79
VIII[e]		105/40	120/92	11.5	2.3	6.53	3	100
IX[f]		36/25	120/140	157	6.6	2.569	$COCH_2$	100
X[h]		55/50	140/100	5.1	1.0	9.27 8.25 8.18 8.11 7.69 7.62	2 2' 6' 6 3'+5' 4'	25 19 12 14 19 11

[a]Using tritiated water of specific activity 5Ci cm^{-1} (10^{-2}cm^3) unless otherwise stated [b]S=substrate, C=catalyst [c]Chemical shifts in d_6-DMSO unless otherwise stated [d]Solvent : pyridine (0.3cm^3) [e]Tritiated as free amine above m.p. [f]Using tritiated water of specific activity 25Ci cm^{-1} (3 x 10^{-3}cm^3) [g]Chemical shift in d_6-benzene [h]Using tritiated water of specific activity 3.8Ci cm^{-1} (5 x 10^{-3}cm^3) : solvent 1,4-dioxane (0.2cm^3)

The final example, 4-phenylpyrimidine (X), provided another example where inter-ring tritium competition could be envisaged. The end result was that all but one position were tritiated so that with tritiated water of higher specific activity, very high specific activities could be potentially obtained.

It was interesting to note that with all the polymer supported catalysts used that the products were invariably of high radiochemical purity, which in due course may be seen as another advantage of these kind of catalysts.

When a comparison was made of the relative efficiencies of all four catalysts, using the deuteriation of 1,4-dimethoxybenzene as the standard reaction, significant differences were observed (Table 4). The results were highly reproducible and showed that the Nafion catalyst led to the best incorporation of label- the degree of deuteriation per unit functionalisation is the best measure of the relative effectiveness of each catalyst. The results can be interpreted in terms of the high acidity of the sulphonic acid groups, accentuated by the highly electronegative nature of the perfluorinated polymer backbone; this more than compensates for the low degree of functionalisation.

In the case of the gel type resins, reducing the average particle diameter by a factor of four nearly doubles the catalyst's effectiveness. This provides clear evidence of the importance of diffusion control in these exchange reactions. Pre-treatment of the Dowex resin in order to remove any traces of water clearly avoids the possibility of isotopic dilution and hence poor incorporation of the label. Finally, the macroreticular resins, which were designed for non-aqueous use, showed reasonable activity with the higher surface area variant Amberlyst 1010 proving to be the most effective by a factor of three.

These experiments serve to show the conditions under which polymer supported acid catalysts can be used to tritiate and deuteriate a number of structurally different organic compounds and the factors that are important in obtaining the best incorporation of label.

EXPERIMENTAL

Materials The substrates were purified prior to use, either by distillation or recrystallisation and their purity checked by nmr spectroscopy. The cation exchange

resins were available commercially (Aldrich, BDH). Complete conversion to the H^+ form was ensured by stirring in warm 3M HCl, followed by washing with water until a neutral pH was obtained. All of the resins were finally dried over anhydrous phosphorous pentoxide at 50°C under high vacuum for 24 hours.

Table 4 Comparison of Polymer Supported catalysts in the deuteriation of 1,4-dimethoxybenzene[a].

Catalyst	Surface Area (m^2/g)	Loading (meq/g)	Time (h)	Deuteriation[b] (%)	Deuteriation/ Loading(g/meq)
Nafion	0.8	0.9	72.0	29.9	33.2
Nafion	0.8	0.9	72.0	35.0	38.7
Nafion	0.8	0.9	144.0	62.3	69.2
Dowex 50W-X8 (pre-dried)	100 mesh	4.8	72.0	40.5	8.4
Dowex 50W-X8 (pre-dried)	400 mesh	4.8	72.0	63.8	13.3
Dowex 50W-X8 (as received)	400 mesh	4.8	72.0	3.8	0.8
Amberlyst 15	45	4.3	72.0	21.0	4.9
Amberlyst 15	45	4.3	72.0	22.0	5.1
Amberlyst 1010	540	3.3	72.0	51.3	15.5

[a]Reactions conducted at 100.0°C in 1,4-dioxane. [1,4-dimethoxybenzene] = 1.25 mol dm^{-3}, [D_2O]=5.88 mol dm^{-3}, [catalyst] = 310g dm^{-3}. Reaction scale = 0.5cm^3.

[b]Calculated from ^1H nmr signal integrals. Deuteriation confirmed by recording ^2H nmr at 46MHz with broad band proton decoupling.

Hydrogen Isotope Exchange Reactions In a typical reaction, substrate (10-50mg), solvent (0.1-0.5cm^3) if required and polymer supported catalyst (20-100mg) were introduced into a thick walled (5mm o/d) pyrex tube and a small amount (see Tables) of tritiated water of the appropriate specific activity was added from a microsyringe. The tube was frozen, evacuated, flame sealed and placed in a thermostat at the required temperature for a pre-determined time. On completion the tube was cooled, cut open, and the solution was removed, injected into diethyl ether (10cm^3) and washed with cold water (3 x 10cm^3) in order to remove labile tritium. The organic layer was separated, dried over anhydrous $MgSO_4$, and the solvent removed by passing a stream of N_2 over the surface.

The tritium incorporation and specific activity were ascertained by preparing solutions of the substrate of known concentration and counting aliquots using a Beckman LS 1800 Liquid Scintillation Counter.

The regiospecificity of tritiation was determined by recording ^{3}H nmr spectra on a Bruker AC300 FT NMR Spectrometer operating at 320MHz with broad band proton decoupling. Chemical shifts were ghost referenced to ^{3}H-tetramethylsilane. Radio-gas chromatograms of liquid samples were obtained using a Carlo-Erba 4200 Chromatograph equipped with a polarised ion chamber detector. In the case of solid samples, radiopurity data was obtained by radio-thin layer chromatography using a Berthold LS200 scanner.

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REFERENCES

1. See for example, Isotopes in the Physical and Biomedical Sciences (Buncel, E. and Jones, J.R. eds), Vol 1. Labelled Compounds (Part B), Elsevier, Amsterdam, 1991.
2. Evans E.A., Warrell D.C., Elvidge J.A. and Jones J.R.-Handbook of Tritium NMR Spectroscopy and Applications, Wiley, 1985.
3. Williams, P.G.-in Isotopes in the Physical and Biomedical Sciences (Buncel, E. and Jones, J.R. eds), Vol 2. Isotopic Applications in NMR Studies, p55, (Part B), Elsevier, Amsterdam, 1991.
4. Garnett J.L., Long M.A., Vining R.F.W. and Mole T.-Tetrahedron. Lett., 4531 (1976).
5. Hanzlik R.P., Wiley R.A. and Gillesse T.J.-Tetrahedron. Lett., 16: 523 (1979).
6. Yavorsky P.M. and Gorin E.-J. Am. Chem. Soc. 84: 1071 (1962)
7. Sherrington, D.C. and Hodge, P. (eds), Syntheses and Separations using Polyfunctional Polymers, Wiley, Chichester, 1988.
8. Brewer, J.R, Jones, J.R., Kawrie, K.W.M., Saunders, D. and Simmonds, A.-J. Chem. Soc. Chem. Commun., 1566, 1990.
9. Culbert, P.A. and Hunter, D.H.-Reactive Polymers, in press, 1993.

10. Kabalka, G.W., Green, J.F. and McCullum, G.-7th Int. Symp. Radiopharm. Chem., Groningen, The Netherlands, p90, 1988.

11. Bean G.P, Brignell P.J., Johnson C.D., Katritzky A.R., Ridgewell B.J., Tarhan H.O. and White A.M.-J. Chem. Soc. (B) 1222 (1967)

12. Bressel U., Katritzky A.R. and Lea J.R.-J. Chem. Soc. (B) 4 (1971)

13. Brewer, J.R.-PhD Thesis, University of Surrey, 1992.

14. Simmonds, A.-private communication.