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Tritium Labelled Alkenes *via* the Shapiro Reaction

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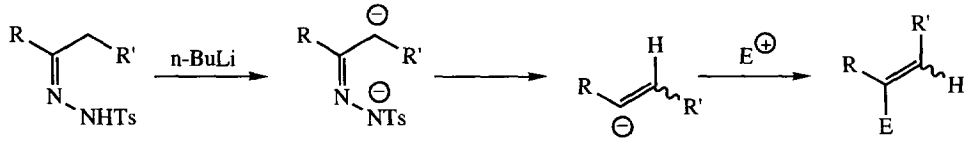
Abstract: We report a simple synthesis of a variety of tritiated alkenes with high specific activity. In each case, the trisylhydrazone derivative of the ketone was converted to the vinyllithium intermediate, which was then quenched with high specific activity tritiated water to generate the corresponding tritiated alkene. The specificity of tritium labelling was determined by tritium NMR spectroscopy.

We have adapted the Shapiro reaction to give tritium labelled alkenes at high specific radioactivity. Trisylhydrazone derivatives were prepared from the appropriate ketone and trisylhydrazide under acidic conditions. The labelling steps involved *in situ* generation of the vinyllithium derivative of the intermediate trisylhydrazone at low temperature, followed by quenching with high specific activity tritiated water. Hence, for substrates which give clean products in the Shapiro reaction, the use of high specific activity tritiated water as an electrophile is a facile approach to producing tritiated alkenes. The stereochemistry of the tritiated alkene may be analyzed by tritium and proton NMR spectroscopy.

The reaction between an aliphatic tosylhydrazone, containing an α-hydrogen, and an alkyllithium to yield alkenes was first reported by Shapiro.[1] This procedure was later modified by allowing the vinyl anion intermediate to be trapped by an electrophile,[2] as shown in the following scheme:



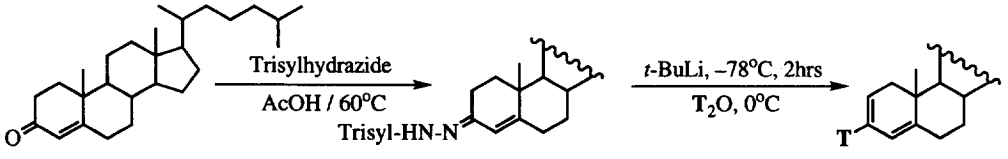
The procedure has been applied to deuterium labelling of a number of arenesulfonylhydrazones under various reaction conditions, and the extent of deuterium labelling has been studied.[3] There are two preparative shortcomings[2b] of the Shapiro reaction which greatly reduce the effectiveness of quenching with isotopically labelled water (i). partial protonation of the dianion by the solvent and (ii). ortho-metallation of the tosyl ring by the organolithium base. For example, we prepared the tosylhydrazone derivative of 4-phenylcyclohexanone by standard procedures and the vinyl anion was trapped using deuteriated water as the electrophile. In spite of a high chemical yield, combined ^{1}H and ^{2}H NMR and mass spectrometric analyses showed only 14% deuterium incorporated at the desired position.

The use of triisopropylbenzenesulfonylhydrazone (trisylhydrazone) derivatives in the Shapiro reaction has alleviated many of these shortcomings.[4] With these derivatives the dianion intermediate can be formed at –78°C, a reaction solvent such as THF can be used, the need for a large excess of base is eliminated, and ortho-metallation is impossible.[4a] The dianion decomposes to the desired vinyl carbanion much more rapidly than in the case of the equivalent tosylhydrazone, and the enhanced rate of elimination of the trisyl derivative is believed to be due to steric destabilization by the bulky ortho substituents.[4b]

In confirmation of the other limitations of the Shapiro reaction discussed above, we found that using methyllithium in ether to generate the intermediate vinyl anions in THF resulted in very low isotope incorporation in the final alkene. This is due to the partial protonation of the vinyl anion from the ether solvent,[5] and the use of *tert*-butyllithium in pentane as the organolithium base alleviated this problem.

There are very few direct methods for the synthesis of tritiated alkenes. The most common approach is tritiation of a suitable acetylenic precursor in the presence of a deactivated palladium catalyst, such as Lindlar's catalyst.[6a-d] Generally this procedure gives the *Z* alkene with high incorporation of tritium (*i.e.* specific activity *ca.* 50 Ci mmol^{-1}).[6e,f] However, in a recent report[7] tritiation of 11-tetradecynyl acetate by this approach yielded a mixture of *E* (23.5%) and *Z* (63.1%) isomers in addition to the fully saturated compound (13.4%) from the acetylenic precursor. Clearly, some disadvantages of the Lindlar approach include yielding predominantly a *Z* alkene product, over-reduction to the alkane, and the empirical nature of arriving at appropriate reaction conditions. In addition, conditions determined for the hydrogenation or deuteriation process are not always appropriate for the tritiation reaction. In contrast, the stereochemistry of the alkene product from a Shapiro reaction is determined by a number of factors, including the geometry of the arenesulfonylhydrazone, the solvent, the bulk of the lithiation reagent, and equilibration time for the vinyl carbanion intermediate.[2-4] Predominantly *E* products are possible, as when an *E* :*Z* ratio of 86:14 of isomeric stilbenes was obtained by decomposition of the tosylhydrazone derivative of phenyl benzyl ketone.[8]

Based on current knowledge of the Shapiro reaction[2] we have developed a simple deuterium and tritium labelling technique for alkenes which we have applied to four substrates. The overall scheme is illustrated for the synthesis of cholest-4-en-3-one trisylhydrazone[9] and the corresponding tritiated diene.



From the many substrates investigated using the Shapiro reaction,[2] the following tritium labelling examples were chosen (Table 1):

a). Generation of a tritiated double bond in a six-membered ring ([1-^{3}H]-4-phenylcyclohexene).

b). Synthesis of a tritiated alkene from a large ring ketone ([1-^{3}H]-cyclododecene), furnishing a mixture of tritiated *Z* (36.5%) and *E* (63.5%) isomers as shown by tritium NMR analysis. A previous study using the di-tosyl intermediate[10] also gave the *E* isomer as the major product.

c). The successful preparation of [3-^{3}H]-cholesta-2,4-diene (as illustrated in the Scheme) from the α,β-unsaturated ketone, cholest-4-en-3-one, *via* the trisylhydrazide derivative. Conjugated dienes are difficult to prepare by alternative procedures due to low yields and tedious syntheses.[9]

d). Synthesis of a tritiated compound with a double bond in a constrained five-membered ring 3-methoxy estrone to give the [17-^{3}H] steroid alkene.[11]

e). We also synthesized β-ionone trisylhydrazone and generated the corresponding triene using H_2O as the electrophile, but the product was volatile and therefore not suitable for tritiation studies.

The chemical yield, deuterium and tritium incorporation as well as the specific radioactivity of all the tritiated alkenes studied are given in Table 1. Specific activity values for the poorly-chromophoric alkenes

were determined by a combination of gas chromatography (mass) and liquid scintillation counting (total radioactivity). The specific activity of each steroid product was also calculated from proton and tritium NMR spectra.[12] The specific radioactivity of the products ranged from *ca.* 14-25 Ci mmol^{-1}. Since the maximum theoretical specific activity achievable with one tritium atom per molecule is 28.76 Ci mmol^{-1}, 50-86% of the product molecules contained a tritium atom.

Table 1: ^{2}H and ^{3}H Labelled Alkenes *via* the Shapiro Reaction

^{2}H Alkene	Yield %	%D	δ [a] (ppm)	^{3}H Alkene	Yield %	S.A. Ci/mmol	%T	δ [a] (ppm)
[1-^{2}H]-4-Phenyl cyclohexene	45	80	5.70	[1-^{3}H]-4-Phenyl cyclohexene	82	24.7 [c]	86	5.70
[1-^{2}H]-Cyclododecene	74	93	5.33 (E) 5.27 (Z)	[1-^{3}H]-Cyclododecene	71	22.4 [c]	78	5.38 (E) 5.32 (Z)
[3-^{2}H]-Cholesta-2,4-diene	68	91	5.87	[3-^{3}H]-Cholesta-2,4-diene	83	23.5 [d]	82	5.86
[17-^{2}H]-Estrone Alkene	66	56	5.90 [b]	[17-^{3}H]-Estrone Alkene	86	14.3 [e]	50	5.93

a) Benzene-D_6 solvent, b) THF-D_8 solvent, c) GC/LSC measurements d) Determined by GC/LSC and tritium NMR spectroscopy, e) Determined by radio-HPLC and tritium NMR spectroscopy.

The tritiation technique was as follows: trisylhydrazone (23-33 mg, 0.05 mmol) was placed in a side-arm flask on a vacuum line and rigorously evacuated. The flask was then brought to *ca.* 75 kPa with nitrogen gas, dry THF (300 μL) was injected and the mixture was stirred for 2 minutes. The reaction temperature was lowered to –78°C (dry ice:acetone), *t*-BuLi (1.7 M in pentane, 65 μL, 0.11 mmol, 2.2 eq.) was added and the mixture was stirred at this temperature for 2 hours to form the dianion. The reaction was then warmed to 0°C (dry ice:water) until nitrogen evolution ceased (*ca.* 5 minutes, the vinyl anion is generated).[3,4] Highly tritiated water (0.1 mmol, 2 μL, *ca.* 5.7 Ci, 1-1.2 equiv.) was prepared prior to the experiment from platinum oxide and carrier free tritium gas in a separate flask,[13] and was injected into the reaction mixture as a THF solution (0.2 mL), at 0°C. After 5 minutes stirring at room temperature, the THF was evaporated, and methanol (1 mL) was added and removed under vacuum. The vessel was then disconnected from the vacuum line, and the residue was extracted with hexane (2 mL). TLC (hexane:acetone-95:5) was used to confirm the identity of the product. The crude reaction mixture was passed through a small silica gel column and the desired compound was eluted with the same solvent system. After drying under a stream of nitrogen, the product was dissolved in deuterated solvent for liquid scintillation counting, GLC, HPLC and NMR analyses. An aliquot of deuterated samples was taken for mass spectrometric analysis.

In conclusion, tritiated alkenes can be simply produced from ketones containing α-hydrogens in good chemical yield, with high specific radioactivity and radiochemical purity, using a microscale synthesis. We believe this reaction is a useful method for the synthesis of tritiated alkenes, especially steroids, and the use of trisylhydrazone derivatives in dry THF maximizes isotopic incorporation. Labelled alkenes with two

prochiral centers are also useful intermediates for cycloaddition reactions (*e.g.* Diels-Alder) and for the synthesis of the corresponding epoxy compounds followed by reduction with hydrides to generate chiral secondary alcohols. We can also envisage using other tritiated electrophiles[3,4] in place of tritiated water for quenching the reaction intermediate (*e.g.* tritiated CH_3I), with the possibility of very high specific activity products.

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