

Preparation of ^2H - and ^3H -Labelled Carvone

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SUMMARY

(-)-Carvone labelled with ^2H or ^3H at C-10 was prepared by two methods. The first, involving a reversible ene reaction yielded 10-deuteriocarvone with some substitution at other reactive centres. An improvement to this route involved the decomposition of an organozinc reagent of 10-chlorocarvone which gave a better yield of product substituted only at C-10. As a preliminary to a possible radioimmunoassay with the above material, four derivatives of carvone linked to bovine serum albumin were prepared.

Key Words: Tritium and deuterium-labelled carvone, synthesis, radioimmunoassay

INTRODUCTION

(-)-Carvone (1) is the principal constituent of the essential oil of *Mentha spicata* L. and is of widespread occurrence as a minor component in the oil of many other herbs: it is a compound of some commercial importance. We set out to synthesise labelled carvone of sufficient activity for use in a radioimmunoassay designed to select high-yielding cell clumps from tissue cultures of *Mentha spicata* with a view to establishing a clone of productive cells. Only reactions which substituted a tritium label in the 10-methyl group were investigated as this position should be inert under the conditions of radioimmunoassay and is a convenient target carbon. Deuterium was used in the developmental stages.

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RESULTS AND DISCUSSION

Our first route was a modification of an earlier synthesis¹ which utilised a reversible ene-reaction to incorporate the label. First an adduct of carvone with *N*-sulfinyl-*p*-toluenesulfonamide (2: freshly prepared from *p*-toluenesulfonamide, $pTsNH_2$, and thionyl chloride²) was formed. Addition of a large excess of deuterium oxide to the reaction flask exchanged most of the amine protons for deuterium, and these atoms could then exchange at C-10 by a retro-ene reaction (Figure 1) in a one-pot process. Free (2) liberated in this process may either be hydrolysed to $pTsNH_2$ and SO_2 (in which case no further reaction occurs) or may survive to form a new adduct and thus repeat the cycle.

The deuteriocarvone product (3) was isolated in 25% yield and was almost optically pure (94% enantiomeric excess). 1H -NMR spectroscopy showed a diminished intensity in the signal from the 10-methyl group, and decoupled ^{13}C -NMR revealed a triplet at the C-10 position due to coupling between ^{13}C and 2H , as well as a signal due to unlabelled carvone. (The ^{13}C assignments of the 7- and 10-methyl groups were independently established by a (1H - ^{13}C)-heteronuclear correlation experiment).

Mass spectroscopy showed that the product contained 62% 2H_1 , 25% 2H_2 and 12% undeuteriated components, whilst the fragment ions suggested that the second label was not always at C-10 (i.e. was not entirely located by recycling of (2)). 2H -NMR spectroscopy demonstrated that some of these additional deuterium atoms were at the enolizable 3-position. The 3β axial position, *cis* to the isopropylidene group was preferentially substituted, as expected by kinetic arguments.

Thus, this method produces carvone labelled with one and sometimes two deuterium atoms at the 10-methyl group, but also introduces label at an enolizable position.

An alternative method, based on the decomposition of an organozinc derivative, proved more suitable for our purposes. Thus, 10-chlorocarvone³ (4) was synthesized in good yield (70%) from (-)-carvone

Table 1 : NMR spectra of deuteriated-carvone*

Position	$\delta^{13}\text{C}$ [50 MHz]	$\delta^1\text{H}$ [200 MHz]	$\delta^2\text{H}$ [22 MHz]
1	146.7*	-	-
2	199.8	-	-
3	43.1	2.35 dd (J=16.0, 13.4 Hz) [3 β] 2.55 ddd (J=16.0, 3.7, 1.6 Hz) [3 α]	2.35 2.55
4	42.5	2.65 m	-
5	31.3	2.25 dddq (J=18.3, 10.8, 2.5, 2.5 Hz) [5 β] 2.45 ddddq (J=18.3, 5.9, 4.5, 1.6, 1.5 Hz) [5 α]	- -
6	144.4	6.77 m	-
7	15.8	1.78 dd (J=2.5, 1.5 Hz)	-
8	135.4*	-	-
9	110.3	4.81 s and 4.76 s	-
10	20.5	1.75 s	1.75
	20.2 t (J=19 Hz)		

* Assignments of C-1 and C-8 uncertain. All other carbons were assigned from proton spectrum. Proton assignments were made from consideration of coupling constants.

+ ¹³C and ¹H spectra ran in [²H]-chloroform; ²H spectrum ran in chloroform.

and Ca(OC1)₂. The organozinc reagent formed from this halide was then decomposed in the presence of deuterio-trifluoroacetic acid to yield 10-deuteriocarvone (Figure 2). Protection of the C=O group was not necessary as it is not implicated by this Zaitsev-type reaction.

Deuterio-trifluoroacetic acid was chosen as a source of label both because it contains no enolizable protons to interfere with exchange, and as it can be easily formed from the practically instantaneous hydrolysis of trifluoroacetic anhydride with deuterium oxide. All the label is then available for transfer (unlike the situation with water, where only one proton can be transferred per molecule); and indeed if the reacting quantities are correctly adjusted, it should be possible to transfer all the label. The reaction was optimized for several solvents (THF, dioxan and ether⁴), with zinc prepared in different ways (acid-washed and "activated"⁵) and for varying periods of time with different concentrations of reagents. Acid-washed zinc gave poor yields (<10%).

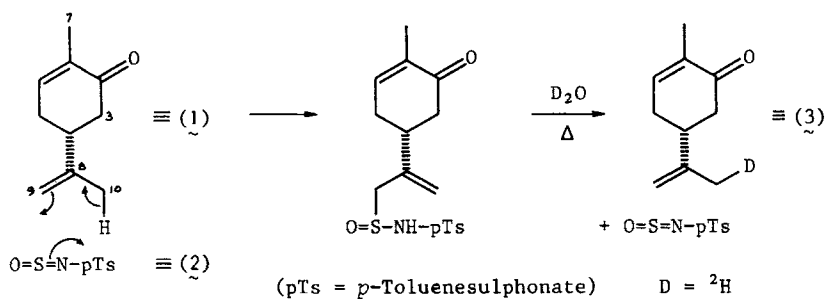


Figure 1

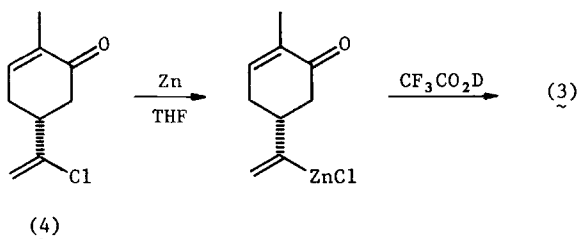


Figure 2

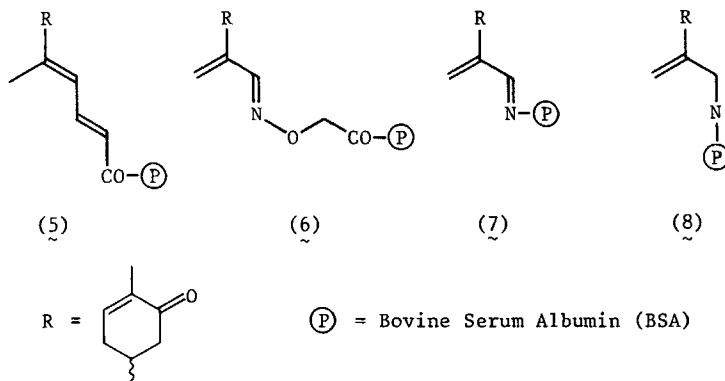


Figure 3

"Activated" zinc was much better (up to 80% yield), and reaction was normally complete within 3 hours. THF was the best solvent. With deuterium as label, the product was almost entirely mono-deuteriated (as indicated by the mass spectrum) and all the label was at C-10 (via ^2H -NMR). Hence this was chosen as the method of choice for preparation of ^2H -labelled carvone.

The reaction of 10-chlorocarvone with tritiated trifluoroacetic acid proceeded in good yield (71%). The specific activity of the product was 5.4 mCi/mmol, which represents 12% of the activity of the tritiated water (5 Ci/ml) used to produce labelled trifluoroacetic acid. This primary isotope effect ($k_H/k_T = 8:1$) could presumably have been avoided if the reacting quantities had been more carefully adjusted to be equimolar. The labelled carvone maintained substantial optical activity (86% enantiomeric excess) and would be suitable for use in radioimmunoassay if a greater atom excess of ^3H in [^3H]-water were to be used.

In order to thus use this labelled material, protein-linked adducts incorporating "spacers" have to be prepared as antigens. Four such adducts were prepared (5)-(8) by standard couplings⁶. The epitope densities were 15:1, 7:1, 10:1 and 3:1 (all $\pm 10\%$) as determined by UV-difference spectroscopy, and in the first example the value was confirmed by the increase in mass of the adduct as shown by SDS-PAGE. As a density of 10:1 to 30:1 is optimal for radioimmunoassay, the first adduct should be well suited for such studies.

EXPERIMENTAL

NMR spectra: were recorded on Varian XL 200 and VXR 400 MHz machines. ^2H spectra were recorded (at 22MHz), unlocked, in CHCl_3 with traces of [^2H]-chloroform present as reference. ^1H - ^{13}C correlations were established using a standard HETCOR pulse sequence.

Mass spectrum (2) m/z (rel. int.) 152 (3.55) [M^+2]; 151 (6.96) [M^+1]; 150 (1.35) [M^+]; 136 (1.44) [150-Me]; 135 (1.27) [151-Me]; 109 (36.4); 82 (100).

Preparation of (3): All apparatus was thoroughly dried. (-)-Carvone (4.5g; ex Aldrich) was added to a stirred solution of (2) in sodium-dried benzene (14.0g/100ml). After 24 hours, [$^2\text{H}_2$]-water (15 ml; 99.8 atom % excess) was added and the mixture was refluxed for 7 hours. White solid was removed from the cooled solution by vacuum filtration, the supernatant was taken up in Et_2O (100 ml) and separated from the aqueous

layer. The organic layer was washed successively with water and brine, then dried (MgSO_4) and reduced to a yellow oil on rotary evaporation. Extraction of this oil into pet. spirit (b.p. 60-80°C)/ Et_2O (4:1 v/v) removed residual pTsNH_2 , and carvone (>99% pure by GC) was obtained by prep. GC (column: silicone oil 10% on Chromasorb W AWDVS 80-100 mesh; 4M x 1/8 in. stainless steel; temp. prog.: 150°C-250°C at 10°C min^{-1}).

10-Chlorocarvone: A suspension of $\text{Ca}(\text{OCl})_2$ (41.2 g; 70% available Cl_2) in water (80 ml) was added to (-)-carvone in CH_2Cl_2 (46.6g/600 ml) and the mixture kept cool by the periodic addition of solid CO_2 over two hours. Vigorous mechanical stirring was essential for good yields. The two-phase reaction was allowed to warm for 1 hour, then vacuum-filtered and the organic layer dried. Distillation under reduced pressure (2 cycles; 1.0 mm Hg; 110-115°C) yielded 10-chlorocarvone (>99% pure by GC).

[^3H]-Trifluoroacetic acid: Trifluoroacetic anhydride (311 mg) was added dropwise to [^3H]-water (27 mg; 5 Ci/ml, ex. Amersham PLC). The product was used immediately.

[^3H]-Carvone: Care was taken to ensure all reagents and solvents were dry, and again all apparatus was thoroughly dried (at 100°C) before use. Freshly-cut potassium metal (220 mg) was added slowly to a stirred solution of ZnCl_2 (403 mg) in THF (30 ml) under N_2 , and then brought to reflux for 3 hours to generate a black suspension of zinc metal ("activated" zinc). A few drops of 10-chlorocarvone (dried over CaCl_2) in THF, were added to the warm solution 5 minutes before the bulk of the 10-chlorocarvone (520 mg total) was added as a mixture with [^3H]-trifluoroacetic acid (338 mg in 0.5 ml THF). The reaction was kept at reflux for 3 hours, then cooled, filtered from unreacted metal under vacuum, and taken up in water (50 ml). This was extracted with Et_2O (3 x 50 ml) and the combined organic layers washed with water (50 ml). The organic layer was then dried (MgSO_4) and the solvents removed at the water pump. Column chromatography on silica gel-H with CHCl_3 :toluene (1:1 v/v) separated carvone ($R_f=0.42$) from oligomeric and reduced by-products. A second column eluted with EtOAc :n-hexane (1:3) ($R_f=0.38$),

removed unreacted 10-chlorocarvone and yielded carvone (>99% pure by GC). The activity of the tritiated product was determined by LSC using "Aquasol" scintillation cocktail (New England Nuclear). Counting efficiency was 24.5% by sample channels ratio and external standards ratio and at least 10⁶ disintegrations were collected (2σ±0.6%).

ACKNOWLEDGEMENT

We thank the S.E.R.C. for funding G.D.B

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