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SYNTHESIS OF 4- AND 10-DEUTERATED NERYL AND GERANYL-β-D-GLUCOSIDES AND THEIR USE IN CORROBORATION OF A MECHANISM PROPOSED FOR THE FRAGMENTATION OF HETEROSIDES IN TANDEM MASS SPECTROMETRY

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SUMMARY

In order to corroborate a mechanism proposed for the fragmentation of the molecular ion of heterosides, involving a hydride migration from the aglycone to the osidic unit, the 4-[$^2H_2$]-10-[$^2H_3$]-labelled neryl and geranyl-β-D-glucosides **9** and **10** have been synthesised.

Deuterated ketone **1** was prepared in >99% isotopic abundance by base catalysed exchange with [$^2H_2$]-water, and was reacted under Wittig-Horner conditions furnishing the corresponding α, β-unsaturated esters **3** and **4**.

Selective reduction of the ester group can be performed with DIBAl-H, whereas $LiAlH_4$ give a secondary reduction of the C=C double bond.

The published procedure (13) for the β-D-glucosidation of alcohols has been modified in order to optimise conditions on the deuterated nerol and geraniol.

By comparison of collision spectra (NICI/CAD) of pure deuterated and undeuterated neryl and geranyl-β-D-glucosides the proposed fragmentation mechanism is fully corroborated.

KEY WORDS : deuterated nerol ; deuterated geraniol ; deuterated labelling ; Wittig-Horner reaction ; β-D-glucosidation ; low energy CAD spectra (NICI/CAD).

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INTRODUCTION

Some natural derivatives of volatile compounds were identified in various vegetables (1), particularly heterosides of volatile alcohols (monoterpenols, aliphatic or aromatic alcohols) from various fruits : grapes (2), apricot (3), papaya (4) and Passion fruit (5).

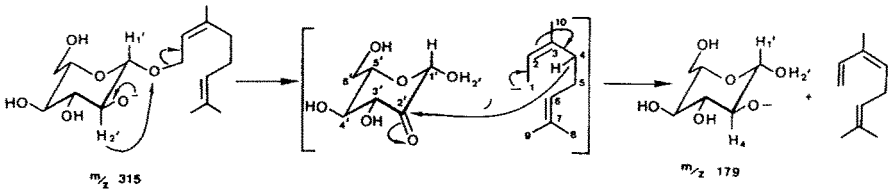
These precursors of volatile compounds are present at levels which are often greater than those of the free volatile alcohols. They thus constitute an unexploited potential aroma which might be liberated by the action of osidases. These enzymes are more or less specific since their mode of action may depend on the nature of the aglycone.

In order to select the osidase system able to achieve the specific osidic bound cleavage it is necessary to elucidate first the exact structure of the precursors.

We first developed analytical methods based mainly on HPLC or soft ionisation mass spectrometry (6). The positive mode of desorption/chemical ionisation allowed mass determination concurrently with the identification of the nature and linking of the different units constitutive of the heterosides. However no information on isomerism in the aglycones can be obtained by this technology.

Tandem mass spectrometry, which allows the differentiation of isomers (7), has been used to analyse qualitatively the isomeric monoterpenyl-β-D-glycosides, particularly geranyl, neryl or linalyl-β-D-glucosides (8,9).

All the daughter and grand daughter ions formed in the collision activated dissociation of molecular ion $(M-H)^-$ arise from the osidic part, whereas the aglycone is eliminated as a neutral fragment. It must be pointed out that the relative abundance of these ions is dependent on the nature and structure of the aglycone. This dependence could be explained by the intermediate formation of an anionic ketonic complex in which hydride migration could take place more or less readily from aglycone to osidic unit.



Scheme 1 - *Example of formation of the daughter m/z 179 $(C_6H_{11}O_6)^-$ ion from the m/z 315 $(M-H)^-$ ion of neryl-β-D-glucoside.*

For neryl and geranyl-β-D-glucosides the allylic hydrogens in positions 4 and 10 would have the greatest ability to migrate. Therefore, in order to corroborate our assumption we have synthesised the 4-[$^2H_2$] - 10-[$^2H_3$]-labelled geranyl and neryl-β-D-glucosides **9** and **10**.

EXPERIMENTAL

Gas Chromatography

GC-analysis were performed on a DB5 capillary colomn using the following analytical conditions : H_2 as carrier gas (1.2 ml/min), H_2 as burning gas (30 ml/min), air (300 ml/min) and additional N_2 (3 ml/min); heating programme from 60°C to 100°C at 2°C/min rate and then from 100°C to 250°C at 10°C/min; detection by flame ionisation.

Infrared

The IR-spectra were recorded on a 377 Perkin-Elmer spectrometer in CCl_4 solutions.

^{1}H-NMR

The ^{1}H-NMR (60MHz) spectra of ketones, esters and alcohols were recorded on EM-360 Varian spectrometer in $CDCl_3$ solutions with TMS as internal standard.

The ^{1}H-NMR (250 MHz) spectra of monoterpenyl glucosides were recorded on a AC250 Brucker spectrometer in CD_3OD solutions.

Mass spectrometry (MS and MS/MS)

All mass spectrometry experiments (MS and MS/MS) were performed on a triple quadripole mass spectrometer (Nermag R-30-10). NICI was used with NH_3 as reagent gas introduced in a modified high pressure source. All spectra were obtained using a direct DCI probe where 1 µl of sample solution (0.2 µg/µl) was placed on the tungsten filament. The source operating conditions were : emission current 130 mA; repeller voltage, 0 V; source temperature, 150°C and ammonia pressure, 9.10^{-5} Torr. The low energy CAD spectra of the selected parent $(M-H)^-$ ion were obtained using argon at 9.10^{-6} Torr (multiple collision conditions) at 10 eV as E_{lab}. The scan rate was 0.5 s for each recorded spectrum by using PDP 11/73 (with a SIDAR system). Each reported spectrum is an average of 60 consecutive scans [from $(M-H)^-$ formed in NICI] and about 10 scans from ions produced by DCI probe heating.

6-Methyl-1-[$^2H_3$]-3-[$^2H_2$]hept-5-ene-2-one **1** :

A solution of 2.4 ml (16.2 mmol) undeuterated 6-methylhept-5-ene-2-one and 2g (14.3 mmol) potassium carbonate in 20 ml [$^2H_2$]-water was stirred at reflux for 4h. After cooling the mixture was extracted by diethyl ether (3 x 20 ml). The combined organic layers were dried over anh. Na_2SO_4, filtered and evaporated. The deuteration process was repeated twice and the residue was purified by chromatography on silica gel column (20 g) by eluting with hexane-diethyl ether (80/20, V/V).

TLC : Rf = 0.2 (hexane-diethyl ether : 90/10, V/V).

GC : T_R = 19 min.

IR (CCl_4, 0.2 M) :

2960 s, 2920 s, 2860 m, 1715 vs, 1440 s, 1410 s, 1355 s, 1270 m, 1220 m, 1180 m, 1150 s, 1110 m.

^{1}H-NMR ($CDCl_3$) [δ (ppm), J (Hz)] :

6-Methyl-hept-5-ene-2-one :

1.66 and 1.63 (2s, 6H, $(CH_3)_2C=$), 2.13 (s, CH_3CO), 2.20-2.55 (m, 4H, CH_2CH_2), 4.82, 5.16 (m, 1H, CH=C).

6-Methyl-1-[$^2H_3$]-3-[$^2H_2$]hept-5-ene-2-one **1** *:*

1.66 and 1.63 (2s, 6H, $(CH_3)_2C=$), 2.26 (d, 2H, $COCD_2CH_2$, $^3J_{HH}$ = 8), 5.06 (t, 1H, C=CH, ^3J = 8).

***Ethyl-3,7-dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(E)-6-eneoate* 3 *and Ethyl-3,7-dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(Z)-6-eneoate* 4 :**

A solution of 28 ml (6.46 mmol) ethyl (O,O'-diethyl phosphoryl) acetate in 10 ml anhydrous THF was added under nitrogen at 0°C to a dispersion of 0.285 g (7.1 mmol) sodium hydride in 10 ml anhydrous THF. After 12h stirring at 0°C, 0.87 ml (5.87 mmol) deuterated 6-methyl-hept-5-ene-2-one was added with stirring at 0°C, and the solution stirred for 12h at 20°C (11). After distillation of THF under vacuum the residue was dissolved in 50 ml water and the solution extracted with CH_2Cl_2 (3 x 50 ml); the organic layer was dried over Na_2SO_4, filtered and the solvent evaporated. The two isomeric esters were then chromatographically separated on a silica gel column (40 g) by eluting with hexane-diethyl ether (99/1, V/V).

Ethyl-3,7-dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(E)-6-eneoate **3** :

Eb = 246°C/248 Torr

Elemental analysis :

$C_{12}H_{20}O_2$: % Calc. C 73.47 H 10.20

% Found C 73.87 H 10.38

TLC : Rf = 0.36 (hexane-diethyl ether : 90/10, V/V)

GC : T_R = 22.5 min.

IR (CCl_4, 0.2 M) (undeuterated ester) :

2980 vs, 2915 vs, 2910 w, 2860 m, 1710 vs, 1645 vs, 1480 w, 1460 w, 1445 s, 1390 w, 1380 s, 1375 s, 1365 s, 1350 s, 1320 m, 1300 w, 1270 w, 1260 w, 1220 vs, 1140 vs, 1110 s, 1100 m, 1060 s, 1040 s, 985 w, 960 w, 890 w, 870 m, 850 w.

^{1}H-NMR ($CDCl_3$) [δ (ppm), J (Hz)] :

1.30 (t, 3H, O-C-CH_3, ^3J = 7), 1.66 and 1.73 (2s, 6H, $(CH_3)_2C=C$), 2.00-2.30 (m, 2.7H, CD_3-C-CD_2CH_2), 4.18 (q, 2H, O-CH_2, ^3J = 7), 4.90-5.25 (m, 1H, $CH=C(CH_3)_2$), 5.66 (s, 0.5H, =CH-CO).

Ethyl-3,7-dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(Z)-6-eneoate **4** :

Eb = 240°C/248 Torr.

Elemental analysis :

$C_{12}H_{20}O_2$: % Calc. C 73.47 H 10.20

% Found C 73.67 H 10.18

TLC : Rf = 0.42 (hexane - diethyl ether : 90/10, V/V)

GC : T_R = 24 min.

IR (CCl_4, 0.2 M) (undeuterated ester) :

2980 vs, 2925 s, 2915 s, 2860 m, 1710 vs, 1650 vs, 1480 w, 1460 w, 1440 s, 1390 w, 1375 s, 1350 w, 1320 w, 1290 w, 1240 s, 1210 s, 1155 vs, 1135 s, 1100 m, 1060 s, 1035 m, 985 w, 910 s, 860 m, 730 m.

^{1}H-NMR ($CDCl_3$) [δ (ppm), J (Hz)] :

1.26 (t, 3H, O-C-CH_3, ^3J = 7), 1.66 and 1.73 (2s, 6H, $(CH_3)_2C$=), 1.83 (s, 0.3 H, CH_3-C=), 2.13 (d, 2H, =C-CD_2-CH_2, ^3J = 8), 4.15 (q, 2H, OCH_2, ^3J = 7), 5.16 (t, 1H, C=CH, ^3J = 8), 5.66 (s, 0.5H, =CH-CO-).

***3,7-Dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(E)-6-ene-1-ol* 5 *and 3,7-dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(Z)-6-ene-1-ol* 6 :**

Reduction by $LiAlH_4$:

The reaction was performed under a nitrogen atmosphere. A solution of 1 g (5 mmol) ester in 20 ml dry diethyl ether was added dropwise, at -20°C, over 30 min., to a stirred solution of 0.8 g (20 mmol) $LiAlH_4$ in 20 ml dry diethyl ether. After 10 min. further stirring the excess of hydride was destroyed by the addition of ethyl acetate. The mixture was poured into a 10% H_2SO_4 solution in ice-water. After extraction with diethyl ether, the organic layer was neutralized with an aqueous 10% solution of sodium hydrogenocarbonate and washed with water to neutral pH. The solution was dried on anhydrous Na_2SO_4, filtered and the solvent evaporated. The alcohols were chromatographically purified on a silica gel column by eluting with hexane-diethyl ether (70/30, V/V).

Reduction by $LiAlH_4/AlCl_3$ (3/1) :

The reaction was performed under a nitrogen atmosphere. To a solution of 3.96 g (10.4 mmol) $LiAlH_4$ in dry diethyl ether cooled in an ice-bath, 0.47 g (3.5 mmol) $AlCl_3$ was added portionwise with caution (violent reaction). The mixture was brought over 1h to room temperature, whilst maintaining stirring 0.51 g (2.6 mmol) ester in 4 ml dry ether was added with stirring over 1h to the reaction mixture. After 40 min., 6 ml ether and 5 ml of a saturated aqueous NH_4Cl solution were added. The mixture was then filtered on glass-wool, dried over anhydrous Na_2SO_4, filtered and the solvent distillated under vacuum. The residual alcohols were purified as already described.

Reduction by DIBAl-H :

The reaction was performed under nitrogen atmosphere. To a solution of 0.51 g (2.6 mmol) ester in CH_2Cl_2, 10.5 ml of 1 M solution of DIBAl-H in CH_2Cl_2 was added slowly, maintaining the temperature lower

than 30°C by cooling with ice-water bath. After 12h stirring at room temperature, a solution of 1 g methanol in 5 ml CH_2Cl_2 and then 0.6 g water in 5 ml methanol were added cautiously. The solvent was evaporated and the residue partitioned between 20 ml CH_2Cl_2 and 20 ml water. The organic layer was decanted; the water was extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were dried over Na_2SO_4, filtered and the solvent evaporated (12). The remaining alcohols were purified as already described.

3,7-Dimethyloctadi-2(E)-6-ene-1-ol (Geraniol) :

TLC : Rf = 0.15 (hexane-diethyl ether : 70/30)

GC : T_R = 19 min.

IR (CCl_4, 0.2 M) :

3620 s, 3340 m, 2960 vs, 2920 vs, 2880 vs, 2860 vs, 2730 w, 1665 s, 1440 vs, 1380 vs, 1375 vs, 1350 m, 1330 w, 1220 w, 1160 w, 1110 w, 1090 m, 995 vs, 960 m, 920 v, 830 m.

^{1}H-NMR ($CDCl_3$) [δ (ppm), J (Hz)] :

1.56-1.83 (m, 10H, $(CH_3)_2C=$, CH_3-C=, OH), 2.00-2.16 (m, 4H, CH_2CH_2), 4.16 (d, 2H, CH_2O, ^3J = 8), 5.00-5.26 (m, 1H, $(CH_3)_2C=CH$), 5.43 (t, 1H, =CH-CO, J = 7).

3,7-Dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(E)-ene-1-ol **5** :

^{1}H-NMR ($CDCl_3$) [δ (ppm), J (Hz)] :

1.66 and 1.73 (2s, 7.2H, $(CH_3)_2C=$, $CD_3C=$), 2.10 (d, 2.3H, CH_2-$CD_2C=$, J = 8), 4.03-4.26 (m, 2H, OCH_2), 4.93-5.23 (m, 1H, $(CH_3)_2C=CH$), 5.43 (t, 0.5H, =CH-C-O, ^3J = 8).

3,7-Dimethyloctadi-2(Z)-6-ene-1-ol (Nerol) :

TLC : Rf = 0.15 (hexane-diethyl ether : 70/30, V/V)

GC : T_R = 17.2 min.

IR : (CCl_4, 0.2 M) :

3600 s, 3420 m, 2960 vs, 2920 vs, 2860vs, 1660 m, 1440 vs, 1380 vs, 1350 m, 1320 w, 1260 w, 1140 w, 1110 w, 1080 m, 1040 w, 985 vs, 960 m, 910 m, 830 m.

^{1}H-NMR ($CDCl_3$) [δ (ppm), J (Hz)] :

1.30 (s, 1H, OH), 1.60 and 1.70 (2s, 6H, $(CH_3)_2C=$), 1.76 (s, 3H, CH_3-C=), 1.90-2.16 (m, 4H, CH_2CH_2), 4.05 (d, 2H, OCH_2, ^3J = 8), 4.90-5.20 (m, 1H, $(CH_3)_2C=CH$), 5.43 (t, 1H, =CH-CO, ^3J = 8).

3,7-Dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(Z)-6-ene-1-ol **6** :

^{1}H-NMR ($CDCl_3$) [δ (ppm), J (Hz)] :

1.26 (s, 1H, OH), 1.66 and 1.76 (2s, 6H, $(CH_3)_2C=$), 2.06 (d, 2.3H, $CH_2CD_2C=$, J = 8), 4.00-4.20, (m, 2H, OCH_2), 5.10 (t, 1H, $(CH_3)_2C=CH$, J = 8), 5.46 (t, 0.5H, =CH-C-O, ^3J = 8).

Neryl and geranyl-β-D-glucosides **10** *and* **9** :

They were synthesised by a modification of Ishag et al. (13), the reagent being mixed in the following proportions, 0.05 g (0.32 mmol) terpenol, 0.263 g (0.64 mmol) α-*tetra*acetobromoglucose, 0.355 g (4.8 mmol) *tertio*-butanol, 0.19 g (0.82 mmol) freshly prepared silver oxide, 2.5 g dry calcium sulfate and 20 ml anhydrous diethyl ether.

The peracetylated glucoside was chromatographically purified on a silica gel column by elution successively with hexane-ethyl acetate (80/20, V/V) and then hexane-ethyl acetate (50/50, V/V).

The deacetylation of peracetylated β-D-glucosides was performed according to the method of Paulsen et al. (14). The product of the deacetylation was chromatographically purified on silica gel column by eluting successively with CH_2Cl_2-CH_3OH (95/5, V/V) and then CH_2Cl_2-CH_3OH (60/40, V/V).

^{1}H-NMR (CD_3OD) [δ (ppm), J (Hz)] :

Geranyl-β-D-glucoside (aglycone part) :

1.65 (s, 6H, CH_3-C=C-C-C-C(CH_3) =), 1.55 (s, 3H, CH_3-C=), 2.04-2.20 (m, 4H, CH_2CH_2), 4.25-4.40 (m, 2H, =C-CH_2-O), 5.10-5.20 (m, 1H, $(CH_3)_2$C=CH), 5.32-5.38 (m, 1H, =C-CH).

Geranyl-4-[$^2H_2$]-10-[$^2H_3$]-β-D-glucoside **9** :

1.55 (s, 3H, CH_3-C=), 1.65 (s, 3.25H, CH_3-C=C-C-C-C(CD_3)=), 2.10 (d, 2.3H, CH_2CD_2-C=, ^3J = 8), 4.18-4.34 (m, 2H, CH_2O), 5.05-5.12 (m, 1H, C=CH-), 5.32 (t, 0.5H, =CH-C-O, ^3J = 8).

Neryl-b-D-glucoside (aglycone part) :

1.55 and 1.65 (2s, 6H, $(CH_3)_2$C=), 1.75 (s, 3H, CH_3-C=C-C-O), 2.02-2.14 (m, 4H, CH_2CH_2), 4.18-4.34 (m, 2H, CH_2O), 5.07-5.12 (m, 1H, C=CH), 5.32-5.38 (m, 1H, =CH-C-O).

Neryl-4-[$^2H_2$]-10-[$^2H_3$]-β-D-glucoside **10** :

1.55 and 1.65 (2s, 6H, $(CH_3)_2$C=), 1.75 (s, 0.25H, CD_3-C=C-C-O), 2.05 (d, 2.3H, CH_2CD_2C=, ^3J = 8), 4.13-4.32 (m, 2H, CH_2O), 5.07-5.12 (m, 1H, C=CH), 5.35 (t, 0.5H, =CH-CO, ^3J = 8).

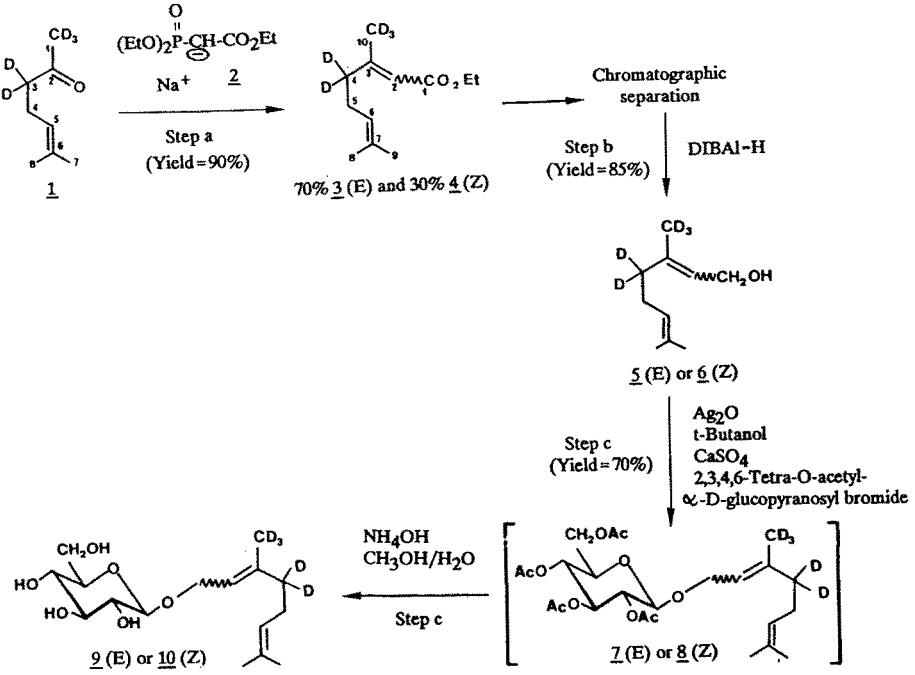
DISCUSSION

Most of the methods described in the literature for the synthesis of nerol and geraniol necessitate many steps and afford very low overall yields, further more they often involve reagents that are difficult to handle such as keten.

Bunton et al. (16) tried to avoid these drawbacks using a Wittig reaction in the synthesis of 4-[$^2H_2$]-10-[$^2H_3$]geraniol **5** and nerol **6** from 6-methyl-1-[$^2H_3$]-3-[$^2H_2$]hepta-6-ene-2-one **1**. But to the best of our knowledge, these authors published neither the experimental conditions nor the yields, and so their

preliminary note is not very useful from a preparative point of view. Further, it must be pointed out that they did not mention several difficult points : first, stabilized Wittig reagents such as 2 are well known to react sluggishly with ketones (17); further, they used $LiAlH_4$ to reduce the esters to the alcohols and secondary reactions can occur in this case as shown by our own results; lastly, no comments are given on the stereoselectivity of their method.

Therefore, we have developped a similar synthesis, based on a Wittig-Horner reaction, which affords the 4-[$^2H_2$]-10-[$^2H_3$]labelled geraniol 5 and nerol 6 in high yields and with high stereoselectivity.



Scheme 2 - *Synthesis of deuterated neryl and geranyl-β-D-glucosides.*

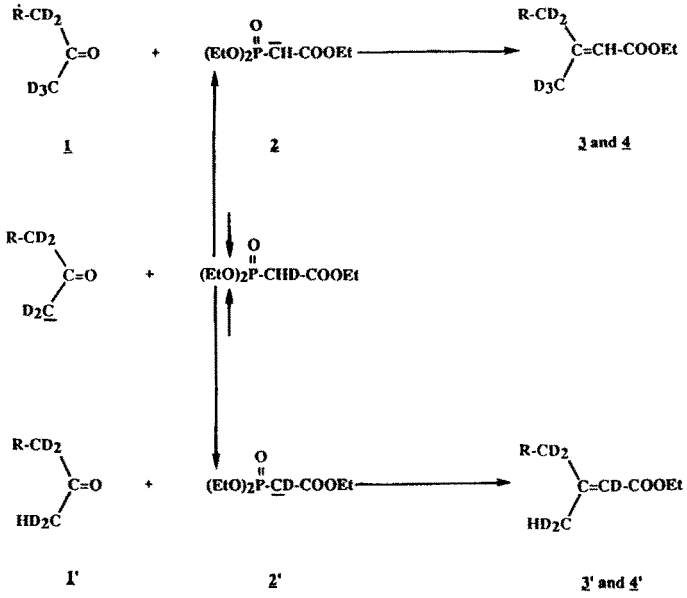
The main features of this synthesis are the following :

Step a. The average extent of deuterium labelling reaches 99 % for each exchanged hydrogen in the 6-methylhepta-5-ene-2-one 1, as determined by ^{1}H-NMR and NICI-Mass spectrometry.

Steps b and c. A mixture of esters 3 and 4 (in a 79/21 ratio as indicated by GC-analysis) was obtained from the Wittig-Horner reaction (11) in a 90 % yield. Their identification as ethyl-3,7-dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(E)-6-eneoate 3 and ethyl-3,7-dimethyl-4-[$^2H_2$]-10-[$^2H_3$]octadi-2(Z)-6-eneoate 4 has been performed by IR and ^{1}H-NMR spectroscopy on the pure compounds obtained after column-chromatography on silica gel.

The assignment of E and Z configurations was based on the δ-values for protons on the 10-(CH_3) and 4-(CH_2) positions, which are known to be higher for the E-isomers, and specially on their subsequent transformation to the corresponding nerol **6** and geraniol **5**.

It must be pointed out that the ^{1}H-NMR spectra of the deuterated esters **3** and **4** indicate clearly an important labelling at the 10-(CH_3) and 4-(CH_2) positions (85-90 %), to be compared to more than 95 % for the same positions in 6-methyl-1-[$^2H_3$]-3-[$^2H_2$]hepta-5-ene-2-one **1**, but at the same time a deuteration near to 50 % on the 2-(CH) position. It is likely that the isotope exchange observed between the 4- and 10-positions on the one hand and the 2-position on the other hand takes place, before the Wittig-Horner condensation, between both enolisable positions in the ketone **1** and the Wittig-Horner reagent **2** through acid-base equilibria as illustrated by scheme 3 for the 2- and 10-positions.



Scheme 3 - *H/D-Exchange process from position 10 to position 2.*

Steps d and d'. In the reduction of esters **3** and **4** three different reducing agents have been used.

$LiAlH_4$ reacts with the mixture of esters **3** and **4** to give in a 75 % yield a mixture of nerol (25 %), geraniol (60 %) and citronellol (15 %). The lack of specificity of this reagent is correlated with the presence of the ethylenic bond conjugated to the ester function. Indeed, the reduction of such an activated C=C bond is a secondary reaction often encountered with $LiAlH_4$ (18), that, peculiarly, Bunton et al. (16) did not comment upon in their own reduction of methyl nerate and geraniate.

The $LiAlH_4$-$AlCl_3$ reagent is more selective towards the same mixture of esters **3** and **4** as only nerol (18 %) and geraniol'(82 %) are produced, but the yield is low (15 %).

Using DIBAl-H, the pure Z or E esters **3** and **4** (deuterated or not) give respectively the corresponding nerol **6** and geraniol **5** in good yield (85 %). These results corroborate the efficency and high selectivity of this reducing reagent, already well documented (12, 19, 20).

Steps e and e'. The β-D-glucosidation of alcohols described by Ishag et al. (13) uses a large excess of alcohol (8/1) over a nearly equimolecular mixture of silver oxide and α-acetobromoglucose. To avoid the loss of deuterated alcohol, we have modified the experimental procedure.

Firstly, we used a small excess (2/1) of alcohol, but the glucoside was obtained in 40 % overall yield, much lower than the yield (85 %) obtained by Ishag. Secondary reactions, such as dehydrations and rearrangements, likely catalysed by the silver moiety, may account for this. Then, to improve the reaction, an excess of alcohol in the ratio previously described by Ishag has been set up again. Therefore, a low reactive tertiary alcohol was added to the reaction mixture maintaining the terpenol/Ag_2O ratio about 1/2.5. So the reaction affords 70 % yield (calculated in regard to the initial monoterpenol) of deuterated neryl and geranyl-β-D-glucosides **10** and **9** , and also 22 % yield (calculated in regard to the initial α-acetobromoglucose) of *tertio*butyl-glucoside.

It is possible that the lowering of yields observed in the case of a low excess of alcohol results from insufficient solvation of the vicinal ester groups, which can then stabilise the intermediate carbocation and inhibit its trapping by alcohol. If this solvation is re-established by adding *tertio*butanol, their neighboring-group participation is disfavoured and the glucosidation reaction is improved in spite of a concurrent reaction in which *tertio*butanol reacts with the glucoside to give a small amont of the corresponding *tertio*butyl-glucoside. Such processes are very similar to those occuring in the Prevost and Woodward methods (21).

The preparation of pure deuterated or undeuterated neryl and geranyl-β-D-glucosides allowed a comparative study of their collision spectra (NICI/CAD) represented in figure 1 (22,23).

Spectral analysis allows to determine the origin of the daughter ions. Both the m/z value 179 for non-deuterated heterosides and also the mass shift of one mass unit for the daughter ion m/z 180 observed for deuterated glucosides allows to identify the ions m/z 179 as non-deuterated Glu~O^- and m/z 180 as monodeuterated Glu~O^-. Additionally, spectral analysis shows that this displacement is observed for 80 % and 70 % respectively of the corresponding deuterated compounds (**10** and **9**). The peak at m/z 179 for 20 % and 30 % corresponding to non-deuterated Glu~O^- is due to a minor transfer of the hydrogen atom from the 5-, 8- or 9-position of the aglycone to the oside unit.

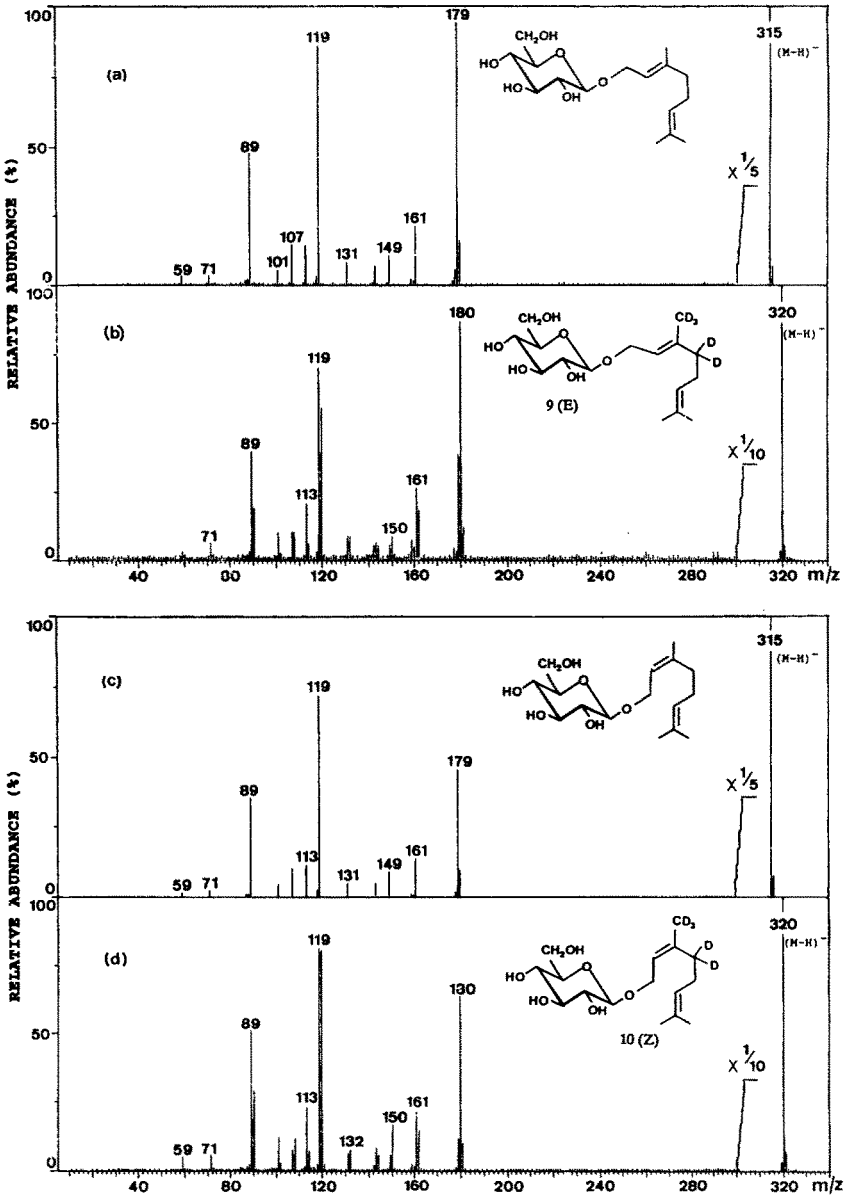


Figure 1 : Low energy CAD spectra of $(M-H)^-$ generated in ammonia NICI from (a) geranyl, (b) 4-[$^2H_2$]-10-[$^2H_3$]geranyl, (c) neryl and (d) 4-[$^2H_2$]-10-[$^2H_3$]neryl-β-D-glucosides.

In conclusion, these results corroborate then the major migration of one deuterium atom from aglycone to glucose part during the CAD process resulting in the formation of m/z 179 ion. The transfer from the positions 4 and 10 is more favoured for the Z-isomer **10** than for the E-isomer **9**, thus their differenciation is possible.

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