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The Synthesis of [^{14}C] labelled Eutypine.

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Summary :

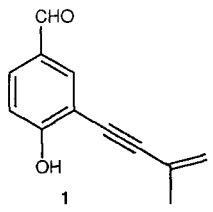
The synthesis of radiolabelled 4-hydroxy-3-(3-methyl-3-buten-1-ynyl) benzaldehyde (eutypine **1**), a phytotoxic compound isolated from the culture media of the fungus *Eutypa lata*, responsible for vineyard die-back, is described. The radioisotope ^{14}C was introduced *via* a Wittig reaction using [^{14}C] methyl-iodide as precursor.

Key words :

[^{14}C] eutypine, *Eutypa lata*, Wittig reaction, vineyard die-back.

Introduction :

Eutypa lata is the pathogenic agent responsible for vineyard die-back (eutypiosis), observed during the last few years in Switzerland and France (1). In the course of our search for secondary metabolites with a phytotoxic activity in the culture medium of *Eutypa lata*, a series of new aromatic compounds with a 3-methyl-3-buten-1-ynyl substituent have been isolated (2, 3). Compound **1** [4-hydroxy-3-(3-methyl-3-buten-1-ynyl) benzaldehyde] named eutypine was found to be the most phytotoxic of compounds isolated (2, 3).



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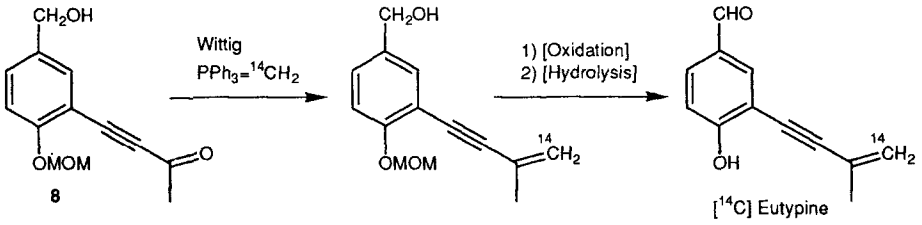
Eutypine **1** and related compounds have been prepared in our laboratory (4) and as part of our research programme (5); an amount of [^{14}C] labelled material was required for metabolism studies. We now describe in this paper the synthesis of [^{14}C] eutypine.

Results and Discussion :

We chose to introduce the radiolabelled carbon at the end of the acetylenic chain by a Wittig reaction with the precursor **8** as shown in scheme 1 :



Scheme 1 : Strategy of the synthesis of [^{14}C] eutypine.

This synthetic approach has the following advantages :

- The position of carbon 14 incorporation is unambiguous.
- The radioisotope is introduced at the final stage of the synthesis.
- [^{14}C] Methyl-iodide used in the Wittig reaction is commercially available.

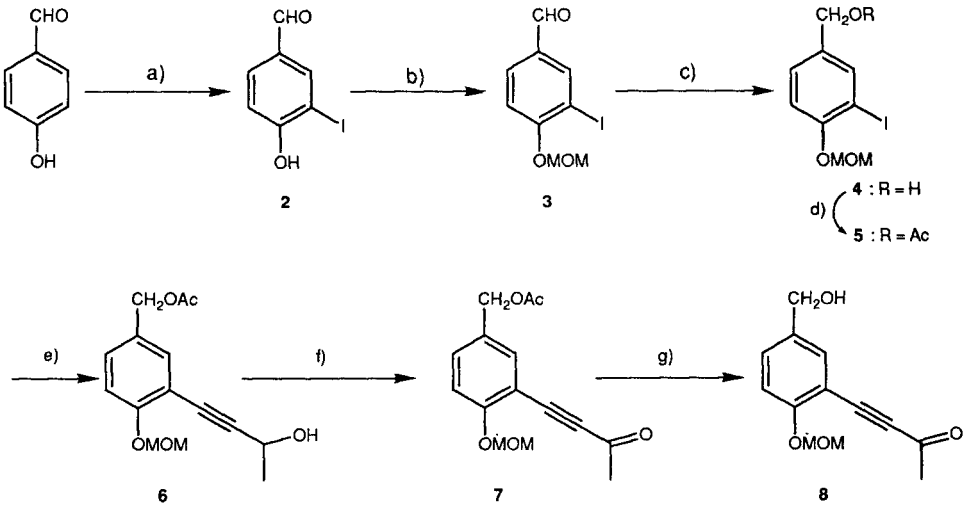
The aldehyde group (on position C-1 of **1**) required protection during the Wittig reaction. As acetal protection was unsuitable, it was decided to reduce the aldehyde group to an alcohol, which was stable during the Wittig reaction and could be easily reoxidized to the aldehyde. The phenolic function was also protected to avoid side reactions. The precursor **8** was prepared in 7 steps from 4-hydroxy benzaldehyde, as depicted in scheme 2.

1° Preparation of the precursor **8** (scheme 2) :

Direct iodination of 4-hydroxy benzaldehyde with I_2/KI in basic media (6) afforded the iodo-compound **2**. The phenolic group was protected as a methoxymethylether (MOM) using dimethoxymethane ($CH_2(OMe)_2$) in acidic medium (7). This protecting group was found to be stable during the coupling reaction. Neither an ester group (as benzoate) nor silyl group (as

TBDMS) were suitable protecting groups (4). Reduction of the aldehyde was achieved using sodium borohydride in methanol and gave the alcohol **4** which was acetylated with acetic anhydride to afford the intermediate iodo-compound **5**.

For the coupling reaction, we used the same conditions as the synthesis of "cold" eutypine (4). Component **5** reacted with the alkyne 1-butyne-3-ol in the presence of a catalyst, palladium bistriphenylphosphine dichloride [$Pd(PPh_3)_2Cl_2$], in diethylamine to give compound **6** in good yield (8). Oxidation of the alcohol group of compound **6** was accomplished using Collins' reagent CrO_3-Pyr_2 (9). It was noted that the acetylenic chain in **7** could not be introduced directly by coupling the iodo compound **5** with 1-butyn-3-one because this alkyne was unstable in the coupling reaction's conditions. Compound **7** was relatively unstable in basic media and so the hydrolysis of the acetate group was performed in mild conditions (K_2CO_3/MeOH aqueous solution) and gave the precursor **8** in satisfactory yield.



a) I_2, KI, $NH(CH_3)_2$ b) $CH_2(OMe)_2$, P_2O_5, CH_2Cl_2 c) $NaBH_4$, MeOH d) Ac_2O, Pyr, CH_2Cl_2

e) 1-butyn-3-ol, $Pd(PPh_3)_2Cl_2$, CuI, $NHEt_2$ f) CrO_3-Pyr_2, CH_2Cl_2 g) K_2CO_3, MeOH/H_2O

Scheme 2 : Synthesis of precursor **8**.

2° Wittig reaction on compound **8** :

The radiolabelled Wittig reagent ($PPh_3=^{14}CH_2$) was prepared by reaction of [^{14}C]-methyl-iodide (1 mCi, 50-60 mCi per mmol) with triphenylphosphine, yielding [^{14}C]

methyltriphenylphosphonium iodide, followed by treament at low temperature with a strong base, *n*-BuLi (10). The precursor **8** was added at 0°C to a solution of the Wittig reagent in THF giving the intermediate **9** which was used in the next step without purification. Treatment of **9** with Collins' reagent gave, after a simple filtration through a silicagel column, the aldehyde. The hydrolysis of the MOM group required mild conditions due to the relative instability of eutypine in acidic media and was therefore performed in AcOH/HCl concentrated solution. [^{14}C] Eutypine was thus obtained in this way in 3 steps from the precursor **8** in 60% overall yield after purification on preparative TLC.

Experimental Part :

All commercially available chemical reagents were used without further purification. [^{14}C]-methyl-iodide was purchased from Amersham. Trial experiments were first performed with unlabelled material. Reactions in the radiolabelled synthesis were monitored by TLC and GC-MS using unlabelled reference compounds. TLC : Aluminium sheets silica gel 60 F_{254} (Merck) ; Preparative column chromatography (CC) : silica gel (Merck 60, 0.063-0.200 mm) ; M.p. : Gallenkamp MFB-595-010M ; ^{1}H-NMR : Bruker AMX 400, chemical shifts in ppm using TMS as internal standard in $CDCl_3$ solutions ; MS (E.I. mode) were recorded on a Nermag R-3010 spectrometer. Microanalyses were performed by the Organic Chemistry Laboratory of ETH Zurich.

4-Hydroxy-3-iodo benzaldehyde (**2**) :

To a solution of commercial 4-hydroxy-benzaldehyde (24.4 g, 0.2 mol) in 30% aq. dimethylamine, an aq. solution of iodine (40.6 g, 0.16 mol) and KI (50 g, 0.3 mol) was added dropwise at room temperature. The reaction was stirred for 3 h. After cooling to 0°C, the mixture was acidified with 10% aq. HCl and extracted with Et_2O. The organic layer was washed with 1% aq. $NaHSO_3$ then with 5% aq. $NaHCO_3$, dried over $MgSO_4$ and evaporated. The crude product obtained was purified by CC (CH_2Cl_2). Yield : 28.8 g (58%). M.p. : 116-117°C ; ^{1}H-NMR : 9.80 (1H, s, C<u>H</u>O), 8.23 (1H, d, ^4J = 1.9 Hz, C_2-<u>H</u>), 7.79 (1H, dd, C_6-<u>H</u>), 7.11 (1H, d, ^3J = 8.3 Hz, C_5-<u>H</u>), 6.50 (1H, br. s, O<u>H</u>) ; MS (M = 248) : $^m/_z$ = 248 (100, $M^{+\cdot}$), 247 (95, M^+-H), 219, 92, 63.

3-Iodo-4-(methoxymethyloxy) benzaldehyde (**3**) :

To a solution of compound **2** (10 g, 0.04 mol) and $CH_2(OMe)_2$ (20 eq., 72 ml) in dry CH_2Cl_2 (100 ml), was added P_2O_5 (35 g) portionwise. After 2 h., more P_2O_5 (20 g) was added. The

mixture was stirred for 5 h. until the disappearance of initial material **2** was observed. The mixture was diluted with CH_2Cl_2, washed with 1N aq. NaOH, then with sat. aq. NaCl, dried over $MgSO_4$ and evaporated. The yellow precipitate obtained was used in the next step without purification, however **3** was purified by CC (CH_2Cl_2) for the spectroscopic data. Yield : 9.3 g (80%). M.p. : 66-67°C ; ^{1}H-NMR : 9.84 (1H, s, C<u>H</u>O), 8.31 (1H, d, C_2-<u>H</u>), 7.82 (1H, dd, C_6-<u>H</u>), 7.17 (1H, d, C_5-<u>H</u>), 5.34 (2H, s, OC<u>H</u>$_2$O), 3.52 (3H, s, C<u>H</u>$_3$) ; MS (M = 292) : $^m/_z$ = 292 (10, $M^{+\cdot}$), 261, 231, 219, 45.

3-Iodo-4-(methoxymethyloxy) benzyl alcohol (**4**) :

To a methanolic solution of compound **3** (5.4 g, 18 mmol) was added portionwise $NaBH_4$ (5 eq., 3.5 g) and the mixture was stirred at room temperature until the disappearance of starting material (1h.) was observed. After concentration under vacuum, Et_2O was added and the organic layer was washed with 5% aq. HCl, then with sat. aq. NaCl, dried ($MgSO_4$) and evaporated. The yellow oil obtained was used in the next step without further purification. Yield : 5.4 g (99%). ^{1}H-NMR : 7.80 (1H, d, C_2-<u>H</u>), 7.27 (1H, dd, C_6-<u>H</u>), 7.04 (1H, d, C_5-<u>H</u>), 5.24 (2H, s, OC<u>H</u>$_2$O), 4.59 (2H, s, C<u>H</u>$_2$OH), 3.51 (3H, s, OC<u>H</u>$_3$), 1.73 (1H, br. s, O<u>H</u>) ; MS (M = 294) : $^m/_z$ = 294 (10, $M^{+\cdot}$), 264, 232, 45.

3-Iodo-4-(methoxymethyloxy) benzyl acetate (**5**) :

The alcohol **4** (3.5 g, 12 mmol) was dissolved in dry CH_2Cl_2 (100 ml) and Ac_2O (10 ml), pyridine (10 ml) and 4-pyrrolidinopyridine (100 mg) were added. The mixture was stirred at room temperature for 3 h. then diluted with CH_2Cl_2. The organic layer was washed sucessively with 5% aq. HCl, 1N aq. NaOH, sat. aq. NaCl, then dried over $MgSO_4$ and evaporated. Compound **5** was purified by CC (CH_2Cl_2). Yield : 3.6 g (90%). ^{1}H-NMR : 7.79 (1H, d, C_2-<u>H</u>), 7.28 (1H, dd, C_6-<u>H</u>), 7.04 (1H, d, C_5-<u>H</u>), 5.24 (2H, s, OC<u>H</u>$_2$O), 4.99 (2H, s, C<u>H</u>$_2$OAc), 3.50 (3H, s, OC<u>H</u>$_3$), 2.09 (3H, s, COC<u>H</u>$_3$) ; MS (M = 336) : $^m/_z$ = 336 (10, $M^{+\cdot}$), 232, 84, 45 ; Anal. calcd for $C_{11}H_{13}O_4I$ (336.13) : C 39.31, H 3.90. Found : C 39.31, H 3.91.

4-(Methoxymethyloxy)-3-(3-hydroxy-1-butynyl) benzyl acetate (**6**) :

The iodo compound **5** (1.45 g, 4 mmol) was dissolved in diethylamine (40 ml) and 1-butyne-3-ol (5 eq., 1.5 g), $Pd(PPh_3)_2Cl_2$ (0.01 eq., 30 mg) and CuI (0.01 eq., 10 mg) were added.

The mixture was stirred overnight at room temperature then acidified with 5% aq. HCl and extracted with Et_2O. The organic layer was washed twice with sat. aq. NaCl, dried over $MgSO_4$ and evaporated. Purification by CC (CH_2Cl_2/EtOAc : 9/1) afforded **6** as a yellow oil. Yield : 1.02 g (85%). ^{1}H-NMR : 7.42 (1H, d, C_2-H), 7.25 (1H, dd, C_6-H), 7.09 (1H, d, C_5-H), 5.24 (2H, s, OCH_2O), 5.00 (2H, s, CH_2OAc), 4.79 (1H, q, CH-CH_3), 3.51 (3H, s, OCH_3), 2.09 (3H, s, $COCH_3$), 1.56 (3H, d, ^3J = 6.6 Hz, CH-CH_3) ; MS (M = 278) : $^m/_z$ = 278 (10, $M^{+\cdot}$), 234, 216, 202, 174, 157, 145, 131, 115, 77, 45

4-(Methoxymethyloxy)-3-(3-oxo-1-butynyl) benzyl acetate (**7**) :

To a solution of compound **6** (2.3 g, 8 mmol) in dry CH_2Cl_2, Collins' reagent CrO_3-Pyr_2 (6 eq., 12.8 g) was added and the mixture was stirred overnight at room temperature under N_2. After dilution with CH_2Cl_2, the mixture was filtered through a short silicagel column to eliminate chromium salts. The solution was then washed sucessively with 5% aq. HCl, 1N aq. NaOH, sat. aq. NaCl, then dried over $MgSO_4$ and evaporated. The crude product was purified by CC (CH_2Cl_2/EtOAc : 9/1). Yield : 1.6 g (70%). ^{1}H-NMR : 7.53 (1H, d, C_2-H), 7.39 (1H, dd, C_6-H), 7.15 (1H, d, C_5-H), 5.27 (2H, s, OCH_2O), 5.02 (2H, s, CH_2OAc), 3.52 (3H, s, OCH_3), 2.47 (3H, s, CH_3), 2.10 (3H, s, $COCH_3$) ; MS (M = 276) : $^m/_z$ = 276 (10, $M^{+\cdot}$), 234, 202, 187, 175, 160, 45 ; Anal. calcd for $C_{15}H_{16}O_5$ (276.29) : C 65.21, H 5.84. Found : C 65.00, H 5.91.

4-(Methoxymethyloxy)-3-(3-oxo-1-butynyl) benzyl alcohol (**8**) :

Compound **8** (1 g, 3.6 mmol) was dissolved in aq. MeOH (1/1 : v/v) and K_2CO_3 (2 eq., 1 g) was added. The mixture was stirred at room temperature for 2 h. then diluted with Et_2O. The organic layer was washed sucessively with 5% aq. HCl, 1N aq. NaOH, sat. aq. NaCl, dried over $MgSO_4$ and evaporated. The yellow oil obtained was purified by CC (CH_2Cl_2/EtOAc : 9/1). Yield : 0.5 g (60%). ^{1}H-NMR : 7.52 (1H, d, C_2-H), 7.39 (1H, dd, C_6-H), 7.14 (1H, d, C_5-H), 5.26 (2H, s, OCH_2O), 4.62 (2H, d, CH_2OH), 3.51 (3H, s, OCH_3), 2.46 (3H, s, CH_3), 2.04 (1H, br. s, OH) ; MS (M = 234) : $^m/_z$ = 234 (5, $M^{+\cdot}$), 192, 160, 131, 45

4-Hydroxy-3-(3-methyl-3-buten-1-ynyl) benzaldehyde (**1**) ([^{14}C] eutypine) :

To [^{14}C] methyltriphenylphosphorane iodide (24 mg, 300 µCi) in dry THF, was added *n*-BuLi (1 eq., 25 µl of 2.4 M solution in hexane) under N_2 and the solution, which became orange,

was stirred for 1 h. at 0°C. Compound **8** (1 eq., 15 mg) in dry THF solution was then added and the mixture was stirred at room temperature for 4 h. The solution was then diluted with Et_2O and washed with 5% HCl, sat aq. NaCl, dried over $MgSO_4$ and evaporated. The crude product obtained was dissolved in dry CH_2Cl_2 and Collins' reagent CrO_3-Pyr_2 (6 eq., 20 mg) was added. After stirring at room temperature for 4 h., the mixture was diluted with CH_2Cl_2, filtered through a short siligagel column and evaporated. The residue obtained was diluted in AcOH (2 ml), a drop of concentrated HCl added and the solution stirred for 1 h. The reaction mixture was then diluted with Et_2O, washed several times with sat. aq. $NaHCO_3$, then sat. aq. NaCl, dried ($MgSO_4$) and evaporated. [^{14}C] Eutypine was purified by preparative TLC (CH_2Cl_2) and obtained as white needles. Yield : 6 mg (60%, 60 μCi).

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