# Bio-supported palladium nanoparticles as a catalyst for Suzuki–Miyaura and Mizoroki–Heck reactions<sup>†</sup>‡

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The biological synthesis of metal nanoparticles from ions has recently emerged as a novel technique for an environmentally benign recovery of heavy metals. Bacteria are known to recover palladium(0) in the form of nanoparticles that are catalytically active. However, the extent of the reactions that can be catalysed by bio-recovered palladium has not been investigated. This study demonstrates that the Suzuki–Miyaura and Mizoroki–Heck reactions can be catalysed by bio-generated palladium nanoparticles formed on the surface of Gram-negative bacteria. The results suggest that the range of applications of this catalyst can be extended to the realm of carbon–carbon bond formation in synthetic organic chemistry.

# Introduction

There is no longer doubt that the development of sustainable chemistry will motivate the emergence of efficient processes to solve current environmental issues for the generations to come. Limitation of natural resources, reduction of wastes, maximising renewability, and development of environmentally benign reagents are the next challenges for the chemical sciences. An important part of modern day chemistry is based on the use of precious platinum group metal (PGM) catalysts. Palladium (Pd), a popular PGM used mainly in catalytic converters, is an expensive metal with a high demand. The price of Pd metal varies concurrently with fluctuating supplies caused by the limited geographical distribution of Pd mines.1 Recovery of waste Pd is therefore of prime importance. Current techniques include costly and non-ecological processes, such as pyrometallurgy, solvent extraction, chemical treatment and electrochemical recovery.<sup>2</sup> Therefore, alternative environmentally benign processes for the recovery of Pd are of high interest.

One such method exploits the capacity of bacteria to reduce transition metal ions like Pd. Certain strains of bacteria (*e.g. Desulfovibrio desulfuricans*,<sup>3-11</sup> *Escherichia coli*<sup>4-11</sup> and *Shewanella oneidensis*<sup>12-14</sup>) can reduce soluble Pd(II) from stock solutions or acid extracts of spent catalysts, forming metallic nanocrystals of Pd(0). This simple protocol involves biosorption of Pd(II) cations on the surface of bacteria and a subsequent reduction to Pd(0) crystals using an electron donor. The nanoparticles formed are supported either on the bacterial outer membrane or in the periplasmic space and remain attached to the cells ("bio-Pd(0)").<sup>14,15</sup> Bio-recovered Pd(0) by Gram-negative<sup>16</sup> and Gram-positive<sup>17</sup> bacteria have been reported to catalyse efficiently hydrogenation reactions,<sup>18</sup> chromium reduction,<sup>8,11</sup> polychlorinated biphenyls (PCB) dechlorination,<sup>14,19</sup> reduction of perchlorate<sup>19</sup> and formation of hydrogen from hypophosphite.<sup>21</sup> Hence, this process for the generation of metal nanoparticles represents a significant step in the direction of developing environmentally friendly catalysts.

In this study, we have explored the possibility of applying bio-Pd for construction of carbon–carbon bonds in synthetic organic chemistry. Furthermore, we have investigated whether the particle size of bio-Pd affects its catalytic properties. Pd(0) has been extensively employed to catalyse various C–C bond forming reactions such as the Suzuki–Miyaura and the Mizoroki–Heck reactions, allowing the elaboration of complex and valuable structures.<sup>20</sup> We present here the first examples of these types of reactions catalysed by bio-generated Pd nanoparticles. Bio-Pd(0) was formed on cells of two Gram-negative proteobacteria, *Cupriavidus necator* and *Pseudomonas putida*, which promote morphologically different Bio-Pd(0).

# **Results and discussion**

# **Bio-generation of Pd(0)**

The bio-Pd was prepared according to the following protocol. After growth and harvesting of the bacteria (*C. necator* or *P. putida*), an aqueous solution of the Pd(II) source (Na<sub>2</sub>PdCl<sub>4</sub>) was added to the cells suspended in anaerobic 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer followed by the addition of an electron donor (formate). The reduction occurred within hours, whereafter the suspension turned from

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<sup>†</sup> This paper is dedicated to Professor Klaus Bock on the occasion of his
65th birthday.
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light orange to black. The buffer was then removed from the bio-Pd by centrifugation, and the recovered bio-Pd pellet was used directly as the catalyst without further purification.<sup>21</sup>

The bio-Pd generated in the presence of the two strains had distinctly different appearances when observed by visual inspection and by transmission electron microscopy (TEM) (Fig. 1). P. putida furnished a poorly dispersed bio-Pd, forming large aggregates, whereas finely dispersed bio-Pd was obtained in the C. necator suspensions. TEM images revealed that bio-Pd supported by both organisms consisted of Pd particles in different sizes ranging from a few nm to hundreds of nm (Fig. 2), and that the particles were associated with the cell surface (Fig. 1). A general trend was that Pd particles were larger in the poorly dispersed sample with P. putida (Fig. 2). Other studies by Macaskie et al.<sup>15</sup> and De Windt et al.<sup>14</sup> have shown that small Pd particles can be located in the periplasmic space of Gram-negative bacteria. Indeed, in our case, both types of bio-Pd obtained had particles under 10 nm in size (Fig. 2), which corresponds to the width of the periplasmic space.



**Fig. 1** (A) Poorly dispersed bio-Pd(0) obtained from *P. putida*: visual inspection (A1) and TEM images (A2, A3). (B) Finely dispersed bio-Pd(0) generated in the presence *C. necator*: visual inspection (B1) and TEM images (B2, B3).



Fig. 2 Palladium particle distribution calculated from TEM images.

#### Catalytic activity of the bio-Pd(0)

We then set out to test the catalytic activity of the bio-Pd for promoting the Suzuki-Miyaura cross-coupling reaction. The bio-Pd formed in *C. necator* suspensions was examined

first because of the smaller and better dispersed particles. Initially, phenylboronic acid and *p*-iodotoluene were chosen as the coupling partners. Various temperatures, bases (organic or inorganic), solvents and mixtures of solvent were screened in order to establish ideal coupling conditions. The reaction of *p*-iodotoluene with 1.1 equivalent of phenylboronic acid in the presence of Na<sub>2</sub>CO<sub>3</sub> (3 equiv) and tetra-*t*-butylammonium bromide TBAB (2 equiv.) in a solvent mixture of EtOH–H<sub>2</sub>O (2:1) at 50 °C provided a 96% yield of the coupling product (Table 1, entry 2), and these reaction conditions were chosen for the further experiments. The same reaction carried out in the absence of the bio-Pd catalyst did not lead to the formation of the biaryl product.<sup>22</sup>

With this catalytic system in hand, we then examined this coupling reaction with a variety of aryl iodides and phenylboronic acid (Table 1).<sup>23</sup> High yields were obtained with both activated (Table 1, entries 3–8) and nonactivated aryl iodides (entries 9 and 10). Despite extensive experimentation, all attempts to include the aryl bromides as electrophiles failed. In comparison to commercially available palladium nanopowder (< 25 nm), the reactivity of bio-Pd was similar in the case of an aryl iodide, but lower in the case of an aryl bromide (Table 1, entries 9 and 11).

Next, the ability of the bio-Pd to promote the Mizoroki-Heck reaction was studied in the coupling of *n*-butylacrylate with a variety of aryl halides (Table 2). The reaction conditions described by Reetz et al. in their study on the same reaction with commercial Pd nanoparticles were applied (Table 2).23,24 As observed in the Suzuki-Miyaura reaction, both activated and nonactivated aryl iodides underwent successful coupling in good to excellent yields under bio-Pd catalysis (entries 1-10). Gratifyingly, the activated aryl bromides also proved reactive under these conditions though with the addition of TBAB (entries 11-13). Again, control experiments confirmed that no reaction occurred without the presence of the added catalyst. The reactivity of the bio-Pd and the palladium nanopowder was comparable for the aryl iodides (entries 1 and 5). Changing the olefin from acrylate to acrylamide did not affect the reactivity (entries 14 and 15). However, nonactivated aryl bromides or activated aryl chlorides were not sufficiently reactive under the conditions used.

Attention was then turned to the other Gram-negative bacterium, *P. putida*, in order to examine the reactivity of the highly aggregated bio-Pd supported by this organism. The reactivity of the *P. putida* bio-Pd in the Mizoroki–Heck reaction is depicted in Table 3. Comparison with the results obtained from the same reaction catalysed with the bio-Pd from *C. necator* (Table 2, entries 1, 7 and 11) shows that both samples of bio-Pd catalyse these couplings efficiently with the substrates tested, and that particle size is not important in this case. The reactivity of the *P. putida* bio-Pd was also tested in the Suzuki–Miyaura cross-coupling between phenylboronic acids and *p*-iodoanisole, leading to a 100% yield of the biaryl product. However, no conversion was observed for *p*-bromobenzonitrile, which is equivalent to that noted with the *C. necator* bio-Pd (Table 1, entries 9, 11).

The recyclability and the fate of the bio-Pd catalyst was also tested, using *C. necator* as the bacterial strain. Recycling of the catalyst was performed after the coupling of *p*-iodotoulene and phenylboronic acid in the Suzuki–Miyaura cross-coupling.

	$B(OH)_2 + R \xrightarrow{\text{III} AB, Na_2CO_3} I \text{III$	R
Entry	Aryl halide	Yield <sup>b</sup> (%)
1		86
2		96 <sup>a</sup>
3	CI	79
4		84
5	H3COC	97
6	онс	85
7	СНО	100
8	Me-HNOC	97
9	MeO	100° (100)°
10	MeO	89
11	NC Br	0 (54)*

 Table 1
 Suzuki–Miyaura coupling of aryl iodides with phenylboronic acid under *C. necator* bio-Pd(0) catalysis<sup>a</sup>

- 2 mol% bio Dd(0)

**Table 2** Mizoroki–Heck reactions under *C. necator* bio-Pd(0) catalysis<sup>*a*</sup>

$\leq 1 \mod 6$ bio-Pd(0) Na <sub>2</sub> CO <sub>3</sub>				
	R + R <sup>2</sup> DMF, 80°C	, 12-24 h R	$\mathbb{R}^2$	
Entry	Aryl halide	R	Yield <sup>b</sup> (%)	
1		COOn-Bu	97 <sup>c,d</sup> (93) <sup>e</sup>	
2		COOn-Bu	98 <sup>c</sup>	
3	CI-	COOn-Bu	100	
4		COOn-Bu	100	
5	онс-	COOn-Bu	88 (99) <sup>e</sup>	
6	СНО	COOn-Bu	86	
7	MeO-	COOn-Bu	96	
8	MeOOC	COOn-Bu	81	
9	MeOOC-	COOn-Bu	86	
10	Me-HNOC	COOn-Bu	96	
11	NC Br	COOn-Bu	97 <sup>c</sup>	
12	O <sub>2</sub> N-Br	COOn-Bu	88 <sup>c</sup>	
13	F <sub>3</sub> C-Br	COOn-Bu	53°	
14		CONH-t-Bu	81	
15	MeO	CONH-t-Bu	99	

<sup>*a*</sup> Reaction conditions: aryl iodide (0.30 mmol), boronic acid (0.33 mmol), TBAB (0.60 mmol), Na<sub>2</sub>CO<sub>3</sub> (0.90 mmol), bio-Pd(0) ( $\leq 2$  mol%) from *C. necator* in EtOH-H<sub>2</sub>O (2:1, 1.5 mL) at 50 °C for 6–16 h. <sup>*b*</sup> Isolated yields after column chromatography. <sup>*c*</sup> 4-Iodoanisole was stirred at 80 °C. <sup>*d*</sup> 4-Iodotoluene (0.39 mmol) and phenylboronic acid (0.33 mmol) were used. <sup>*e*</sup> < 25 nm palladium powder, CAS-number: 7440-05-3 was used as the palladium source.

The reaction was performed on a scale twice the size of that as indicated in Table 1, and the reaction time for each coupling cycle was 24 h. After each cycle the vial was centrifuged, yielding the catalyst as a pellet. The pellet was washed twice with ethanol before the catalyst was reapplied to a coupling reaction. After the catalyst had been reused 4 times (yield: 91%, 97%, 97%, 95%), no loss of activity was found. The fate of the catalyst was examined by TEM after the catalyst had been used in the first coupling and then after the fourth recycling procedure. While the Table 3 The catalytic activity of the bio-Pd from *P. putida* in the Mizoroki–Heck reaction<sup>*a*</sup>



<sup>*a*</sup> Reaction conditions: aryl halide (0.20 mmol), olefin (0.40 mmol), Na<sub>2</sub>CO<sub>3</sub> (0.50 mmol), bio-Pd(0) ( $\leq 1 \text{ mol}\%$ ) from *P. putida* in DMF (2.0 mL) at 80 °C for 24 h. <sup>*b*</sup> Isolated yields after column chromatography.

bio-Pd did not alter appearance after the first coupling reaction, we found a few cells in the bio-Pd after reusing it 4 times. This was presumably due to cell lysis, although it was difficult to determine whether the palladium was still attached to the cell debris. The bacteria are not viable after formation of bio-Pd, and it would be expected that the cell structure is eventually disrupted. Due to the lack of cell viability, the bio-Pd waste does not need to be handled differently from other Pd wastes.

Finally, we examined the possibility of producing catalytically active bio-Pd from Pd(II) salts from waste materials. The waste material for this study was obtained from a hydrogenation reaction with Pd/C as the Pd source. The Pd containing waste was first submitted to oxidation in aqua regia, whereby all solid Pd materials were transformed into soluble Pd(II).25 After neutralisation of the excess acid, the Pd(II) containing solution was subjected to reduction in the presence of C. necator with formate as the reductant. The bio-Pd was separated from the solution by centrifugation and used as a catalyst in the Mizoroki-Heck reaction of *p*-iodoanisole with *n*-butylacrylate (Scheme 1). The coupling product, n-butyl (E)-3-(4-methoxyphenyl)acrylate, could be furnished in a high 90% isolated yield. Half of the palladium was recovered using this procedure, and we are currently working on increasing the efficiency of the recovery using industrial waste as the palladium source.



Scheme 1 Mizoroki–Heck reaction with bio-Pd(0) recovered from a waste solution.

# Conclusions

In conclusion, we have demonstrated the ability of bio-Pd to catalyse two important carbon–carbon bond forming reactions, namely the Suzuki–Miyaura and Mizoroki–Heck couplings. We

have shown that the bio-Pd catalysis may represent a practical and simple means of sustainable organometallic chemistry. This method represents a green alternative to conventional processes for the recovery of important transition metals from wastes. Further studies are now ongoing to investigate the possibility of applying this approach of recycling and reactivation of Pd to industrial wastes.

# Experimental

## **Preparation of bacteria**

The two bacterial strains used in this article were *Cupriavidus* necator ATCC 43291 and *Pseudomonas putida* ATCC 12633. The strains were grown in 150 mL growth liquid media (5 g L<sup>-1</sup> Peptone, 3 g L<sup>-1</sup> Meat Extract, pH 7) at 30 °C on a shaking table (120 rpm) until an optical density of  $OD_{600}=1$  was reached after approximately 16 h. The cultures were then transferred to 50 mL falcon tubes and harvested by centrifugation (10 min, 4416 rpm), washed in anaerobic MOPS-buffer (20 mM, 3 × 30 mL) and finally suspended in anaerobic MOPS-buffer to an  $OD_{600}=1$ .

#### **Bio-reduction of Pd**

The bacterial cell suspension (10 mL,  $OD_{600}=1$ ) and a degassed solution of Na<sub>2</sub>PdCl<sub>4</sub> (6.80 mM, 0.5 mL) in MilliQ water were added to a sealed glass tube and flushed with nitrogen. After 5 min, a degassed solution of formate (1 M, 0.25 mL) in MilliQ water was added, and the tube was placed on a shaking table overnight at 30 °C giving bio-Pd as black aggregates. The formed bio-Pd was then separated from the solution by centrifugation (10 min, 4416 rpm) and removal of the supernatant.

## **Fixation for TEM**

The bacterial cell suspension after bioreduction (100  $\mu$ L) was added to a sterile eppendorf tube and a 25% aqueous solution of glutaric aldehyde (20  $\mu$ L) was added. The eppendorf tube was shaken and left for 10 min. The solution was hereafter centrifuged (2 min, 14000 rpm), washed with MilliQ water (3x 200  $\mu$ L) and finally diluted in MilliQ water to a total volume of 200  $\mu$ L.

#### 4-Methylbiphenyl (Table 1, entry 2)<sup>26</sup>

4-Iodotoluene (85.0 mg, 0.39 mmol), phenylboronic acid (40.2 mg, 0.33 mmol), bio-Pd from *C. necator* suspensions (10 mg, 7 µmol), Na<sub>2</sub>CO<sub>3</sub> (95.4 mg, 0.90 mmol), TBAB (193.4 mg, 0.60 mmol) were added to a sample vial in a glovebox. EtOH (1 mL) and H<sub>2</sub>O (0.5 mL) were added and the sample vial was fitted with a teflon sealed screwcap and removed from the glovebox. The reaction mixture was heated to 50 °C for 6 h and then cooled to 20 °C. The crude reaction mixture was then filtered through a filter paper and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel using pentane as eluent affording the title compound (53.1 mg, 96% yield) as a colourless solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta_{\rm H}$  (ppm) 7.62 (d, 2H, J = 7.6 Hz), 7.53 (d, 2H, J = 6.4 Hz), 7.46 (t, 2H, J = 7.6 Hz), 7.36 (t, 1H, J = 7.6 Hz), 7.28 (d, 2H, J = 7.6 Hz), 2.44 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta_{\rm C}$  (ppm)

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154.4, 141.3, 138.5, 137.1, 129.6 (2C), 128.8 (2C), 127.13 (2C), 127.11 (2C), 21.2. GCMS calcd for  $C_{13}H_{12}$  [M]: 168. found: 168.

#### Butyl cinnamate (Table 2, entry 1)<sup>27</sup>

Iodobenzene (40.8 mg, 0.20 mmol), n-butylacrylate (51.3 mg, 0.40 mmol), TBAB (64.5 mg, 0.40 mmol), Na<sub>2</sub>CO<sub>3</sub> (53.0 mg, 0.50 mmol) and bio-Pd from C. necator suspensions (3 mg, 2 µmol) were added to a sample vial in a glovebox. DMF (2 mL) was added and the sample vial was fitted with a teflon sealed screwcap and removed from the glovebox. The reaction mixture was heated to 80 °C for 12 h and then cooled to 20 °C; H<sub>2</sub>O was added and the crude reaction was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were washed with H<sub>2</sub>O and brine. The organic phase was dried over MgSO<sub>4</sub>. After concentration in vacuo, the crude product was purified by flash chromatography on silica gel using  $CH_2Cl_2$ -pentane (1:1) as eluent affording the title compound (39.7 mg, 97% yield) as a colourless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta_{\rm H}$  (ppm) 7.68 (d, 1H, J = 16.0 Hz), 7.54-7.52 (m, 2H), 7.39-7.37 (m, 3H), 6.44 (d, 1H, J = 16.0 Hz),4.22 (t, 2H, J = 6.8 Hz), 1.73-1.66 (m, 2H), 1.49-1.40 (m, 2H), 0.97 (t, 3H, J = 7.3 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta_{\rm C}$  (ppm) 167.2, 144.7, 134.6, 130.3, 129.0 (2C), 128.2 (2C), 118.4, 65.6, 30.9, 19.3, 13.9. GCMS calcd C<sub>13</sub>H<sub>16</sub>O<sub>2</sub> [M]: 204, found: 204.

## **Recycling experiment**

4-Iodotoluene (170.0 mg, 0.78 mmol), phenylboronic acid (80.4 mg, 0.66 mmol), bio-Pd from C. necator suspensions (20 mg, 14 µmol), Na<sub>2</sub>CO<sub>3</sub> (190.8 mg, 1.8 mmol), TBAB (386.8 mg, 1.2 mmol) were added to a sample vial in a glovebox. EtOH (2 mL) and  $H_2O$  (1 mL) were added and the sample vial was fitted with a teflon sealed screwcap and removed from the glovebox. The reaction mixture was heated to 50 °C for 24 h and then cooled to 20 °C. The mixture was centrifugated (10 min, 5000 rpm) and the supernatant was removed from the pellet. The pellet was dissolved in EtOH (3 mL) and vortexed. The mixture was hereafter centrifuged (10 min, 5000 rpm) and the supernatant was removed from the pellet. The supernatants were combined and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel using pentane as eluent affording the product 4-methylbiphenyl. To the pellet a new batch of substrates, TBAB, base and solvents were added and the reaction was continued.

#### Preparation and treatment of the waste

The in-house generated waste was prepared from a hydrogenation reaction of (*E*)-3-(4-methoxyphenyl)-*N*-methylacrylamide (431.3 mg, 2.26 mmol) with Pd/C, 10% (120.0 mg, 5 mol%) in a mixture of THF–H<sub>2</sub>O (10mL, 1:1) under an atmosphere of hydrogen for 36 h at room temperature (rt). The solution was concentrated *in vacuo*. In order to obtain a full oxidation of the Pd, aqua regia (6 mL) was added slowly to the flask and the solution was heated with a heat gun until boiling point. The mixture was then left and stirred for 1 h at rt. To be tolerated by the bacteria, the suspension was hereafter cooled to 0 °C on an ice bath and neutralised to pH 7 using conc. NaOH solution. This resulted in a Pd waste solution (0.11 mM, 20 mL). The bacterial cell suspension (5 mL, OD<sub>600</sub>=1, *C. necator*) was added to a sealed glass tube and flushed with nitrogen. Then, a degassed solution of the Pd waste (0.11 mM, 3.8 mL) was added to the resuspension. After 5 min, a degassed solution of formate (1 M, 1 mL) in MilliQ water was added and the tube was placed on a shaking table overnight at 30  $^{\circ}$ C. The glass tube was hereafter centrifuged and the water was removed giving the bio-Pd, which was used without further purification.

## (E)-Butyl-3-(4-methoxyphenyl)acrylate (Scheme 1)<sup>27</sup>

1-Iodo-4-methoxybenzene (46.2 mg, 0.20 mmol), n-butylacrylate (51.3 mg, 0.40 mmol), Na<sub>2</sub>CO<sub>3</sub> (53.0 mg, 0.50 mmol), TBAB (65.4 mg, 0.4 mmol) and bio-Pd<sub>waste</sub> (15 mg, 10 µmol) were added to a sample vial in a glovebox. DMF (2 mL) was added and the sample vial was fitted with a teflon sealed screwcap and removed from the glovebox. The reaction mixture was heated to 80 °C for 24 h and then cooled to 20 °C; H<sub>2</sub>O was added and the crude reaction was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were washed with H<sub>2</sub>O and brine. The organic phase was dried over MgSO<sub>4</sub>. After concentration in vacuo, the crude product was purified by flash chromatography on silica gel using  $CH_2Cl_2$ -pentane (1:1) as eluent affording the title compound (42.0 mg, 90% yield) as a colourless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta_{\rm H}$  (ppm) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ (ppm) 7.63 (d, 1H, J = 15.9 Hz), 7.47 (d, 2H, J = 8.7 Hz), 6.90 (d, 2H, J = 8.2 Hz), 6.31 (d, 1H, J = 15.9 Hz), 4.20 (t, 2H, J = 6.9 Hz, 3.83 (s, 3H), 1.72-1.64 (m, 2H), 1.46-1.41 (m, 2H), 0.96 (t, 3H, J = 7.4 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta_{\rm C}$ (ppm) 167.5, 161.4, 144.3, 129.8 (2C), 127.4, 115.9, 114.4 (2C), 64.4, 55.5, 30.9, 19.3, 13.9. GCMS calcd for C<sub>14</sub>H<sub>18</sub>O<sub>3</sub> [M]: 234, found: 234.

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