Pergamon

Tetrahedron Letters 43 (2002) 2269–2271

TETRAHEDRON LETTERS

# A facile 1,5-rearrangement of β-formylenamides and cleavage of esters catalysed by *Pseudomonas fluorescens*

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Received 15 November 2001; revised 2 January 2002; accepted 14 January 2002

**Abstract**—β-Formylenamides undergo a facile 1,5-rearrangement of their *N*-acetyl groups under the influence of the soil bacterium *Pseudomonas fluorescens* to afford β-acetoxyenones in good yields. Further, the soil microorganism efficiently cleaves steroidal and non-steroidal acetates to alcohols. 

The application of enzymes in microbial transformation reactions is an emerging area in the field of organic chemistry.[1] Although, fluorescent Pseudomonads are the most extensively studied bacterial biocontrol agents for a diverse range of soil-borne diseases,[2] their potential in biotransformation reactions has received little attention, except for recent reports where *Pseudomonas* spp. have been employed for the hydroxylation of nicotinic acid[3] and aromatic compounds.[4] On the other hand, an acyl group is a frequently used protective group for alcohols and there has been continued search for mild and efficient methods for the cleavage of esters employing enzymes[5] and Lewis acid catalysts.[6] A β-formylenamide is an interesting functionality bearing an acetyl group that has been exploited for the syntheses of various steroidal and non-steroidal heterocycles.[7] Our attempts to utilise soil micro-organisms in bio-organic transformations has led to a facile 1,5-rearrangement of the *N*-acetyl group of β-formylenamides as well as ester cleavage catalysed by the *Pseudomonas fluorescens* strain RRLJ 134.[8]

In a typical reaction, a solution of 3-hydroxy-16-formyl-17-acetamido-androst-5,16-diene (**1a**, 0.1 mmol) in methanol (10 ml) was evenly distributed into five 100 ml nutrient broth media[9] containing *P. fluorescens* strain RRLJ 134. The culture was inoculated at ambient temperature (28–30°C) at pH 7.4 with constant shaking for 40 h. After TLC indicated the completion of the reaction, the product was isolated by preparative thin layer chromatography and identified as 16-(*Z*)-acetoxymethylene-epiandrost-5-ene-17-one (**2a**) from its spectroscopic properties.[10] The $^1$H NMR spectrum showed a distinctive singlet at $\delta$ 8.35 for a conjugated olefinic proton and a signal at $\delta$ 2.30 for an acetoxy methyl group. Further evidence was provided by its mass spectrum, which showed a significant ion peak at $m/z$ 339 (M−18) corresponding to facile loss of water typical of *C*-substituted steroids.[11]

Treatment of 3-acetoxy-16-formyl-17-acetamido-androst-5,16-diene (**1b**) with the *P. fluorescens* strain under identical conditions led to product **2a**. However, **1c** under the influence of the microorganism afforded the conjugated enone (**2b**) without cleavage of the 3-benzyloxy group. Similarly, *P. fluorescens* catalysed the 1,5-rearrangement of the alicyclic-β-formylenamides (**1d** and **1e**) affording the corresponding conjugated enones (**2c** and **2d**) in good yields (Table 1).

Concerning the reaction mechanism for the formation of **2a**, it may be that under the influence of the microorganism, the canonical form **A** of **1a** facilitates a 1,5-rearrangement of the *N*-acetyl group to give the imine intermediate **B**, followed by hydrolysis to give the product **2a**. The catalytic role of *P. fluorescens* is evidenced from the fact that no rearrangement of **1a** takes place in the absence of the microorganism. However, the use of strong alkaline conditions led to the normal deacetylated product, 17-amino-16-formyl-3-hydroxy-androst-5,16-diene.

*Keywords*: *Pseudomonas fluorescens*; β-formylenamides; soil microorganism; bio-organic transformation.

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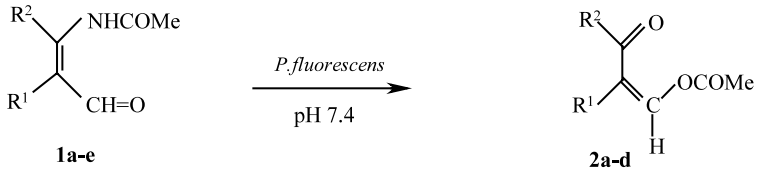
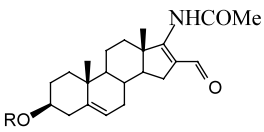
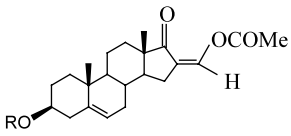
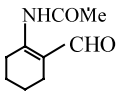
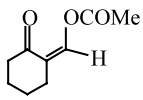
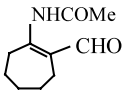
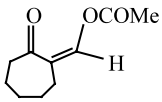
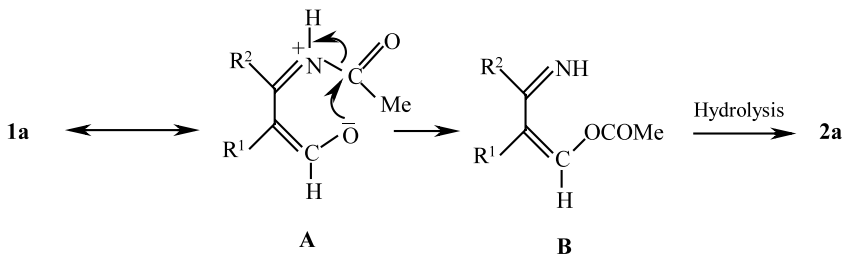
0040-4039/02/$ - see front matter 
PII: S0040-4039(02)00114-4

**Table 1.** Rearrangement reaction of β-formylenamides catalysed by *P. fluorescens*[a]




| Entry | Substrate | Product[b] | Time (h) | Yield[c] (%) |
|---|---|---|---|---|
| 1 |  **1a**, R = H |  **2a**, R = H | 40 | 69 |
| 2 | **1b**, R = COMe | **2a**, R = H | 48 | 68 |
| 3 | **1c**, R = COPh | **2b**, R = COPh | 48 | 55 |
| 4 |  **1d** |  **2c** | 42 | 62 |
| 5 |  **1e** |  **2d** | 44 | 65 |

[a]All reactions were carried out at ambient temperature. [b]Isolated yields. [c]All products gave satisfactory spectroscopic and analytical data.



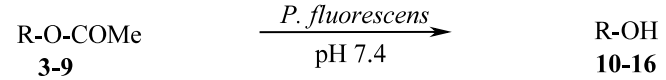

In order to investigate the role of *P. fluorescens* in ester hydrolysis, an attempt was made to treat alicyclic and aromatic acetates with the microorganism under identical conditions. It was observed that cleavage of the acetates (**3**–**9**) with *P. fluorescens* proceeded within 20–28 h to afford the alcohols (**10**–**16**) in high yields (Table 2). The aromatic acetate bearing an electron withdrawing group at the *p*-position (entry 6) proceeded with a faster reaction rate than alicyclic analogues (entries 1–3). However, an attempt to carry out the microbial catalysed cleavage of alicyclic and aromatic benzoates (**3**–**9**, R = Bz) failed in our hands.

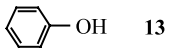
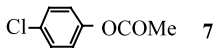
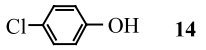
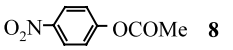
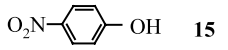
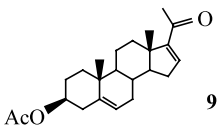
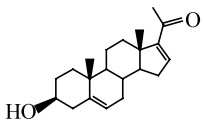
In summary, we have demonstrated that the *P. fluorescens* strain RRLJ 134 efficiently catalyses the 1,5-rearrangement of β-formylenamides to the corresponding β-acetoxyenones in high yields. In addition, the microorganism has proven to be an efficient catalyst for the mild[6] cleavage of acetates. Further work on acetate cleavage using microbial transformation reactions is in progress.

## Acknowledgements

We thank the Department of Science and Technology (DST), Govt. of India, New Delhi for financial support and the University Grant Commission, New Delhi for a teacher fellowship to M.L.

**Table 2.** Hydrolysis of esters catalysed by *P. fluorescens*[a]




| Entry No | Substrate | Product[b] | Time (h) | Yield[c] (%) |
|---|---|---|---|---|
| 1 |  **3** |  **10** | 28 | 72 |
| 2 |  **4** |  **11** | 28 | 74 |
| 3 |  **5** |  **12** | 30 | 68 |
| 4 |  **6** |  **13** | 26 | 80 |
| 5 |  **7** |  **14** | 24 | 82 |
| 6 |  **8** |  **15** | 20 | 83 |
| 7 |  **9** |  **16** | 28 | 88 |

[a]All reactions were carried at ambient temperature. [b]Products were identified by comparison of physical and spectral data with standard samples. [c]Isolated yields.

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8. The strain RRLJ 134 was isolated from the rhizoplanes of tea roots from north-east India and identified as a fluorescent Pseudomonas strain through various morphological, physiological and biochemical tests. The strain maintained in nutrient agar slants was used for study.
9. The broth media is composed of peptone (5 g), sodium chloride (5 g), beef extract (1.5 g) and yeast extract (1.5 g) per litre. The pH of the medium is 7.4.
10. *Selected spectroscopic and analytical data.*
16-(*Z*)-Acetoxymethylene-epiandrost-5-ene-17-one (**2a**): yield 69%; mp 198–99°C (hexane); IR (KBr): $\nu_{max}$ 3391, 2930, 1760, 1660, 1595, 1560, 1427 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 8.35 (s, 1H), 5.40 (bs, 1H), 3.54 (m, 1H), 2.70 (s, 3H), 2.45–1.25 (m, 17H), 1.12 (s, 3H), 1.00 (s, 3H); MS (EI): *m*/*z* 339 [$M^+-H_2O$]. Anal. calcd for $C_{20}H_{30}O_4$: C, 73.71; H, 8.43. Found: C, 73.20; H, 8.02.
2-(*Z*-Acetoxymethylene)cyclohex-1-one (**2c**):[12] yield 62%; mp 52–53°C; IR (KBr): $\nu_{max}$ 2930, 1765, 1660, 1560 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 8.45 (s, 1H), 3.05–1.65 (m, 8H), 2.78 (s, 3H); MS (EI): *m*/*z* 156 ($M^+$). Anal. calcd for $C_9H_{12}O_3$: C, 64.27; H, 7.19. Found: C, 64.10; H, 7.42.
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