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Rapid Communication

Biocatalytic resolution of racemic 6-acetyl-3,4-dihydro-3-hydroxy-7-methoxy-2,2-dimethyl-2*H*-1-benzopyran

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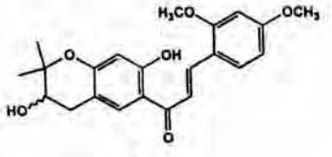
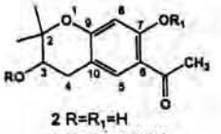
Pseudomonas cepacia lipase catalysed enantioselective acetylation of 6-acetyl-3,4-dihydro-2,2-dimethyl-3-hydroxy-7-methoxy-2*H*-1-benzopyran using vinyl acetate is described.

2,2-Dimethylbenzopyrans and their acyclic analogs, *i.e.* prenylated phenolics are known to possess a wide range of biological activities, such as antibacterial, antifungal, antimicrobial, anticancer, antiulcer and antifeedant.[1-6] These compounds with suitably placed substituent(s)/functional groups could be chiral and the problem of their resolution comes to forefront. Recently, we found that racemic 1-(3,4-dihydro-3, 7-dihydroxy-2, 2-dimethyl-2*H*-1-benzopyran-6-yl)-3-(2,4-dimethoxyphenyl)propenone **1**[7] possesses a strong anti-invasive activity against MCF 7/6 human breast carcinoma cell lines at 100 μ*M* concentration, but was cytotoxic as well.[8] It is well established that one enantiomer of a chiral compound exhibits the desired activity, while the other one is responsible for its cytotoxicity. Therefore, we envisaged the synthesis of **1** and its analogs in optically pure/enriched form. For this, we synthesised its immediate precursor **2**[9] in the racemic form and confirmed its structure by spectral data and X-ray diffraction studies; the crystal structure refinement was done by full matrix least squares on F^2 for all data using SHELXL-96.[10] To resolve the enantiomeric mixture, we tried the biocatalytic transesterification of **2** using enzymes. The initial attempts using *Candida rugosa* (CRL) and porcine pancreatic lipases (PPL) in toluene using vinyl acetate as acyl donor were not successful. The reaction did not proceed beyond 10% conversion even after 20 days. We also attempted the deacetylation of the corresponding diacetate **3**[11] prepared chemically, using PPL in toluene containing *n*-butanol as acyl acceptor; in this case too, no significant progress was observed even after 15 days. These reactions indicated that CRL and PPL are not the enzymes of choice for these substrates (chroman-3-ols). Our attempt to carry out Amano PS catalysed enantioselective acetylation of **2** was quite successful and this is the first report of resolution of chroman-3-ols using lipase, though the resolution of chroman-4-ols (3,4-dihydro-4-hydroxy-2*H*-1-benzopyrans) has been reported using CRL[12] and *Pseudomonas cepcia* lipase(Amano-PS).[13] Patel *et al.*[14] have also demonstrated the stereoselective acetylation of 3,4-dihydro-3,4-dihydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile in toluene using CRL and *Pseudomonas cepacia* lipase. Achiwa *et al.* reported the synthesis of the natural ∝-tocopherol by CRL-catalysed enantioselective hydrolysis of *RS*-tocol acetate[15] and by hydrolysis of DL-∝-tocopherol oxalate[16], in both the cases the enzymatic recognition of a stereogenic carbon atom remote from the reaction site was involved.



1



2 R=R1=H
3 R=R1=COCH3
4 R=H; R1=CH3
5 R=COCH3; R1=CH3

The fact that free phenolic hydroxy group invariably poisons the enzymes prompted us to protect the phenolic -OH in **2** *via* methylation using dimethyl sulphate to obtain 6-acetyl-3,4-dihydro-3-hydroxy-7-methoxy-2,2-dimethyl-2*H*-1-benzopyran **4** in 80% yield. Its stereoselective enzymatic acetylation using Amano PS lipase yielded the acetate **5**, which has not been reported earlier. Both compounds **4** and **5** were well characterized from their spectral data. The compound **4** has been reported earlier as a metabolite[17] but its data is not reported.

Results and Discussion

The chromanol **4** was subjected to Amano PS catalysed enzymatic acetylation using vinyl acetate as the acyl donor in THF at 42-45 °C. The progress of the reaction was monitored by TLC and the reaction quenched after approximately 50% conversion by filtering off the enzyme, the product **5** and the recovered chromanol **4** were separated by flash column chromatography over silica gel. The acetylated product **5** as well as the recovered unreacted chromanol **4** were found to be optically active ($[\alpha]_D$+7.69 and -5.88, respectively), thus indicating that Amano PS catalyses transesterification in an enantioselective manner.

The enantiomeric excess (*ee*) of the recovered alcohol was determined by ^{1}H NMR (400 MHz) spectroscopy using (*R*)-(–)2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) as chiral shift reagent and deuterated chloroform as solvent, *ee* of 50% was obtained. Though other chiral shift reagents, $Pr(FOD)_3$, $Eu(FOD)_3$ and $Eu(DPM)_3$, were also tried, but a clear separation of enantiomers could only be achieved with TFAE. With $Eu(FOD)_3$ and $Eu(DPM)_3$, a downfield shift was seen with peaks appearing as humps with no splitting. For $Pr(FOD)_3$, an upfield shift was seen with humps also. Successful chiral separation could only be achieved for the racemate and the recovered unreacted enantiomer. The *ee* for the acetylated product could not be determined as no splitting was observed with the acetylated enantiomer because there is no binding site left to bind with TFAE. The *ee* was determined by comparing the chemical shifts of the sample alone in deuterated chloroform and the sample plus TFAE (1:1 mole ratio) in deuterated chloroform. The *R/S* ratio could not be ascertained since it was not feasible to differentiate the enantiomers.

Conclusion

To conclude, the present study has shown interesting and potentially useful enantioselectivity during Amano PS catalysed transesterification in an organic solvent; however the reaction did not proceed at all with PPL or CRL. As it is difficult to synthesise these compounds in optically pure form by purely chemical methods, the biocatalytic reaction reported here should find utility in the synthesis of novel optically enriched analogs of different classes of bioactive naturally occurring polyphenolic compounds.

Experimental Section

General. The structure of **2** was established by its X-ray diffraction studies (*cf.* **Figure 1**) *Crystal data*

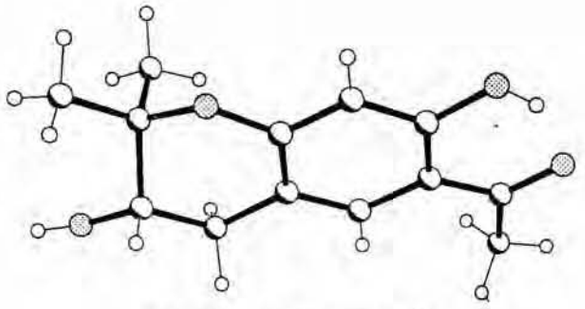


Figure 1 — X Ray structure of **2**

for **2**: Triclinic, space group $P\bar{1}$, a=6.626(5), b = 8.573(6), c = 10.901(7)Å, α = 95.53(6), β = 96.10(6), γ = 104.36(6)°, U = 591.6(7) Å^3, 210(2)K; final R1, wR2 and S are 0.069, 0.203 and 1.005 for 156 parameters. Data were collected using a Siemens R3m four-circle diffractometer in ω-2θ mode to a maximum 2θ 50.1. Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre.

(±)-6-Acetyl-3,4-dihydro-2,2-dimethyl-3-hydroxy-7-methoxy-2*H*-1-benzopyran 4. To a solution of 6-acetyl-3,4-dihydro-3,7-dihydroxy-2,2-dimethyl-2*H*-1-benzopyran **2** (1.0g, 4.27 m*M*) in anhydrous acetone were added anhydrous potassium carbonate (2.0g) and dimethyl sulphate (0.53 cm^3, 1.3 eq), and the solution was refluxed for 5 hr whereupon solvent was evaporated under reduced pressure and ice-cold water (50 cm^3) added to it. The mixture was extracted with ethyl acetate 3 × 100 cm^3) and the combined organic phase dried (Na_2SO_4). After evaporation, the residue was purified by silica gel column chromatography eluting with 10% EtOAc in pet. ether (v/v) to yield the product (0.85g, 80%) as viscous oil; IR (KBr)ν_{max}: 3435, 2977, 2932, 1651, 1613, 1571, 1494, 1468, 1451, 1425, 1360 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$); δ 1.34 and 1.36 (2s, 3H each, 2 × CH_3), 2.55 (s, 3H, $COCH_3$), 2.74(dd, 1H, J = 5.8 and 16.5 Hz, H-4a), 3.05(dd, 1H, J = 4.7 and 16.5 Hz, H-4b), 3.80(t, 1H, J = 5.4Hz, H-3), 3.84 (s, 3H, OCH_3), 6.39(s, 1H, H-8), 7.59(s, 1H, H-5); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 21.74(C-4), 25.06 and 30.27 (2 × CH_3), 31.72 ($COCH_3$), 55.39(OCH_3), 69.26(C-3), 78.06(C-2), 99.96(C-8), 111.16(C-10), 120.88(C-6), 132.84(C-5), 158.04(C-7), 159.65(C-9), 202.3(>C=O); EIMS: m/z 250 ([M])$^+$, 90), 235(40), 217(34), 179(100), 165(45), 147(20), 43(24).

Lipase catalysed resolution of (±)-6-acetyl-3,4-dihydro-3-hydroxy-7-methoxy-2,2-dimethyl-2*H*-1-benzopyran 4. To a solution of **4** (0.5g) in anhydrous

THF (25.0 cm^3) was added vinyl acetate (0.3 cm^3), followed by *Pseudomonas* lipase (Amano PS, 0.50g), and the suspension incubated at 45 °C. The progress of the reaction was monitored periodically by HPLC and TLC examination; after about 50% conversion, the reaction was quenched by filtering off the enzyme. The solvent was removed in *vacuo* and the resulting product column chromatographed when the (+)-acetate **5** eluted out with ethyl acetate-pet. ether (2:23) as a white solid followed by (–)alcohol **4** using ethyl acetate-pet. ether (1:4). Both the compounds were characterized on the basis of spectral data and specific rotations.

Recovered (–)-6-acetyl-3,4-dihydro-2,2-dimethyl-3-hydroxy-7-methoxy-2*H*-1-benzopyran 4: Viscous colourless oil; $[\alpha]_D^{25}$ –5.88° (*c* 0.34, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.34 and 1.38 (2s, 3H each, 2xCH_3), 2.57(s, 3H, $COCH_3$), 2.73 (dd, 1H, J = 5.66 and 16.54 Hz, H-4a), 3.02 (dd, 1H, J = 4.64 and 16.50 Hz, H-4b), 3.83 (t, 1H, J = 5.26 Hz, H-3), 3.85 (s, 3H,-OCH_3), 6.41 (s, 1H,H-8) and 7.62 (s,1H, H-5).

(+)-3-Acetoxy-6-acetyl-3,4-dihydro-7-methoxy-2,2-dimethyl-2*H*-1-benzopyran 5: White solid, mp 96-97 °C; $[\alpha]_D^{25}$ + 7.69 (*c* 0.26, $CHCl_3$); IR(KBr)ν_{max}: 2982, 2936, 1744, 1667, 1614, 1574, 1495, 1467, 1450, 1424, 1387, 1373, 1299, 1283, 1236,1143 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 1.32 & 1.35 (2s, 3H each, 2 × CH_3), 2.05 (s, 3H, $OCOCH_3$), 2.56(s, 3H, $COCH_3$), 2.75(dd, 1H, J = 4.8, 16.9 Hz; H-4a), 3.06(dd, 1H, J = 4.5, 16.7, H-4b), 3.86(s, 3H, OCH_3), 5.03(t, 1H, J = 4.8 Hz, H-3), 6.41(s, 1H, H-8), 7.58(s, 1H, H-5); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 21.00(C-4), 23.09($OCOCH_3$), 24.84(CH_3), 27.34(CH_3), 31.79 ($COCH_3$), 56.45(OCH_3), 70.55 (C-3), 76.25(C-2), 100.04(C-8), 110.33(C-10), 121.21(C-6), 132.55(C-5), 157.72(C-7), 159.63(C-9), 170.34($OCOCH_3$), 197.86(>C=O); EIMS: m/z 292([M^+], 6), 250 (76), 235(37), 217(70), 179(100), 165(63), 147(22), 43(47).

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