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ENZYMATIC ENANTIOSELECTIVE DEACETYLATION STUDIES ON NOVEL (±)-2,4-DIACETOXYPHENYL ALKYL KETONES

Najam A. Shakil,[1] Amarjit Singh,[1] Ashok K. Prasad,[1] Vijayendra Kumar,[1] Carl E. Olsen,[2] Subhash C. Jain,[1] Ashok L. Cholli,[3] Arthur C. Watterson,[3] and Virinder S. Parmar[1,3,*]

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Dedicated to the loving and cherished memory of colleague, friend, and benefactor, Professor Sukant K. Tripathy.

ABSTRACT

Porcine pancreatic lipase pre-incubated with phenyl methyl ketone in THF fails to recognize the *ortho*- and *para*-acetoxy functions with respect to the nuclear carbonyl group towards deacetylation reaction on 2,4-diacetoxyphenyl methyl ketones; this result is in conformity with our hypothesis on the mechanism of action of PPL in THF. Further, four racemic 2,4-diacetoxyphenyl alkyl ketones, the precursors for the synthesis of analogs of a potent antifungal coumarin, 7-acetoxy-4-(1-ethyl)pentyl-3-phenyl-2*H*-1-benzopyran-2-one, have been synthesized by Nencki's reaction between resorcinol and corresponding (±)-aliphatic acid, followed by acetylation of the resulting (±)-2,4-dihydroxyphenyl alkyl ketones. Porcine pancreatic lipase has been used suc-

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cessfully for regio- and enantioselective deacetylation of these diacetoxyphenyl alkyl ketones in tetrahydrofuran.

Key Words: Polyphenolics; Arylalkyl ketones; Lipase; Deacetylation, Regioselective; Enantioselective

INTRODUCTION

Polyphenolics are secondary metabolites of plants and possess an array of biological activities [1-4]. The synthesis of a variety of natural polyphenolic compounds, e.g., coumarins [5], chalcones [6], flavones [7], flavanones [8], chromanones [9], etc. requires polyhydroxyaryl alkyl ketones as substrates. Again, synthesis of enantiomerically pure polyphenolic compounds can be achieved either by the use of enantiomerically pure substrates or by the resolution of the final racemic product. It is at this juncture that the Nature's catalysts "Enzymes" come in handy. The use of enzymes in a synthetic sequence provides unique advantages of efficiency, stereospecificity and environmental friendliness [10, 11]. We have demonstrated earlier, the capabilities of lipases from porcine pancreas (PPL), *Candida rugosa* (CRL) and *Aspergillus* species [12] for regioselective deacetylation of a variety of polyacetoxy aromatic ketones. It has been observed that PPL in tetrahydrofuran (THF) mediates the deacetylation of all other acetoxy groups except those at the *ortho* position(s) to the nuclear carbonyl group in aryl alkyl ketones [13], chalcones [14], desoxybenzoins [15], coumarins and flavanones [16]. To explain the mechanism of selective deacetylation catalyzed by PPL, we postulated that the nuclear carbonyl group present in the substrate forms a transient (dynamic) Schiff's base type complex with the x-amino function of the lysine residue present in the active site of PPL (an analogy to the human pancreatic lipase). The formation of this complex causes the *ortho* acetoxy function to be embedded under the hydrophobic bulk of the active site of the enzyme and the serine-OH takes part in deacetylation of other more suitably placed acetoxy function(s) in the same molecule [17]. However, no direct proof for this mechanism could be given as the active site structure of PPL is not known and also because the Schiff's base formation, being a transient (dynamic) process, could not be verified. Since esters and amides of polyacetoxy aromatic acids can not form Schiff's bases easily as compared to the corresponding ketones, it has been observed by us that incubation of esters and amides of polyacetoxy aromatic acids with PPL leads to the formation of *ortho* hydroxy compounds together with other possible products [18, 19]. These findings substantiate our hypothesis of the presence of lysine in the active site of porcine pancreatic lipase and the formation of the Schiff's base type complexes during deacetylation of peracetates of polyphenolic compounds bearing nuclear carbonyl group.

In continuation, we report herein the deacetylation studies on 2,4-diacetoxyphenyl methyl ketone and (±) 2,4-diacetoxyphenyl (1-phenyl)propyl ketone

catalyzed by PPL which was pre-incubated with phenyl methyl ketone as additional evidence to our dynamic Schiff's base complex formation hypothesis. Further, we report the synthesis of (±)-2,4-diacetoxyphenyl alkyl ketones, precursors of analogs of a potent antifungal coumarin, 7-acetoxy-4-(1-ethyl)pentyl-3-phenyl-2*H*-1-benzopyran-2-one [5] and their enantiomeric resolution employing the regioselective deacetylation capabilities of PPL in THF. The selection of THF as a solvent for these reactions was based on our earlier experiences.

EXPERIMENTAL

Melting points were determined either on a Mettler FP62 instrument or in a sulphuric acid bath and are uncorrected. The UV and IR spectra were recorded on a Cary 100 Biospectrophotometer and Perkin-Elmer model 2000 FT-IR spectrophotometer, respectively. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 and 75.5 MHz, respectively using TMS as an internal standard. The chemical shift values are on d scale and the coupling constants (*J*) are in Hz. Optical rotations were measured on a Bellingham-Stanley AD 220 polarimeter. EI mass and HRMS were recorded on a Jeol AX 505 W instrument at 70 eV. The enzyme, porcine pancreatic lipase (PPL, Type-II) was purchased from Sigma Chemical Co. (USA) and used after storing *in vacuo* over P_2O_5 for 12 hours. THF was redistilled and dried over molecular sieves (4Å). Thin layer chromatography (TLC) was carried out on commercially available Merck silica gel $60F_{254}$ plates. The solvent system used for R_f determination was petroleum ether-ethyl acetate (4:1), the developing agents were either 5% alcoholic $FeCl_3$ solution or iodine vapor. Reactions were monitored at λ_{254} nm on a Shimadzu LC-10AS HPLC instrument with SPD-10A UV-VIS detector and Shimpack CLC-ODS (4.6 × 150 mm) reverse phase column, solvent system used was methanol-water (3:2) at the flow rate of 0.50 ml/min. The chiral ^{1}H NMR shift reagent (+)-TFAE was purchased from Aldrich Chemical Co. (USA).

General Preparation Procedure of (±)-2,4-Dihydroxyphenyl Alkyl Ketones 5-8

The heterogeneous mixture of fused $ZnCl_2$ (2 gm, 15 mmol) and racemic acid (10 mmol) was heated slowly with stirring to make the mixture homogeneous. Resorcinol (1.1 gm, 10 mmol) was added and the reaction mixture was kept for about two hours at 150°C, poured onto crushed ice containing hydrochloric acid (1:1) and extracted with dichloromethane (2 × 50 ml). The organic layer was washed with sodium bicarbonate solution (5%), dried over anhydrous Na_2SO_4 and solvent removed under reduced pressure. The residue thus obtained was purified by column chromatography using petroleum ether:ethyl acetate as eluting solvent to afford racemic 2,4-dihydroxyphenyl alkyl ketones **5-8** in 50-55% yields.

(±)-2,4-Dihydroxyphenyl (1-Phenyl)ethyl Ketone (5)

It was obtained as a viscous oil (1.21g) in 50% yield. R_f: 0.25; IR (nujol): 3343, 2932, 1720, 1629 (C=O), 1509, 1450, 1359, 1230, 1179, 1138, 1063, 979, 946, and 846 cm^{-1}; UV (MeOH): 263, 276 and 317 nm; ^1H NMR ($CDCl_3$): d 1.53 (3H, d, J = 9.1 Hz, C-2'H), 4.62 (1H, q, J = 9.1 Hz, C-1'H), 6.28 (1H, dd, J = 8.8 and 2.4 Hz, C-5H), 6.33 (1H, d, J = 2.4 Hz, C-3H), 7.19-7.34 (5H, m, aromatic protons), 7.67 (1H, d, J = 8.8 Hz, C-6H), and 12.93 (1H, s, chelated OH); ^{13}C NMR ($CDCl_3$): d 20.63 (C-2'), 48.42 (C-1'), 105.08 (C-3), 109.27 (C-5), 114.60 (C-1), 128.55, 128.98, and 130.48 (C-2", C-3", C-4", C-5", and C-6"), 134.20 (C-6), 142.89 (C-1"), 163.92 and 167.23 (C-2 and C-4) and 206.30 (C=O); EIMS, *m/z* (% rel. int.): 242 ($[M]^+$, 20), 227(10), 198(5), 165(5), 137(95), 105(100), 91(10), 81 (55), 53(35) and 39(20).

(±)-2,4-Dihydroxyphenyl (1-Phenyl)propyl Ketone (6)

It was obtained as a viscous oil (1.28 g) in 50% yield. R_f: 0.25; IR (nujol): 3362, 2967, 2930, 2875, 1729, 1630 (C=O), 1512, 1453, 1363, 1270, 1229, 1138, 1029, 999, 970, 905, and 847 cm^{-1}; UV (MeOH): 280 and 318 nm; ^1H NMR ($CDCl_3$): d 0.92 (3H, t, J = 7.3 Hz, C-3'H), 1.81-1.95 (1H, m, C-2'H_a), 2.13-2.25 (1H, m, C-2'H_b), 4.38 (1H, t, J = 7.3 Hz, C-1'H), 6.30-6.35 (2H, m, C-3H and C-5H), 7.28-7.33 (5H, m, aromatic protons), 7.75 (1H, d, J = 8.6 Hz, C-6H) and 12.92 (1H, s, chelated OH); ^{13}C NMR ($CDCl_3$): d 12.66 (C-3'), 27.22 (C-2'), 54.77 (C-1'), 103.99 (C-3), 108.28 (C-5), 114.00 (C-1), 127.52, 128.43, and 129.26 (C-2", C-3", C-4", C-5", and C-6"), 132.98 (C-6), 141.00 (C-1"), 163.37 and 166.25 (C-2 and C-4) and 204.84 (C=O); EIMS, *m/z* (% rel. int.): 256 ($[M]^+$, 25), 213(5), 146(95), 103(20), 91(100), 81(55), 53(35) and 41(40).

(±)-2,4-Dihydroxyphenyl (1-Methyl)propyl Ketone (7)

It was obtained as an oil (1.06 g) in 55% yield. R_f: 0.30; IR (nujol): 3339, 2970, 2936, 2877, 1633(C=O), 1514, 1454, 1383, 1330, 1313, 1232, 1182, 1160, 1133, 1109, 1083, 985, 968, 952 and 846 cm^{-1}; UV (MeOH): 277 and 317 nm; ^1H NMR ($CDCl_3$): d 0.92 (3H, t, J = 7.3 Hz, C-3'H), 1.20 (3H, d, J = 6.8 Hz, CHCH_3), 1.44-1.58 (1H, m, C-2'H_a), 1.76-1.86 (1H, m, C-2'H_b), 3.30-3.39 (1H, m, C-1'H), 6.39-6.43 (2H, m, C-3H and C-5H), 7.68 (1H, d, J = 9.5 Hz, C-6H) and 13.20 (1H, s, chelated OH); ^{13}C NMR ($CDCl_3$): d 12.19 (C-3'), 17.52 (CH*C*H_3), 27.39 (C-2'), 41.73 (C-1'), 104.08 (C-3), 108.33 (C-5), 113.53 (C-1), 132.63 (C-6), 163.40 and 165.99 (C-2 and C-4) and 209.74 (C=O); EIMS, *m/z* (% rel. int.): 194 ($[M]^+$, 10), 163(10), 137(95), 109(25), 81(5), 58(35) and 43(100).

(±)-2,4-Dihydroxyphenyl (1-Methyl)pentyl Ketone (8)

It was obtained as an oil (1.14 g) in 52% yield. R_f: 0.20; IR (nujol): 3345, 2959, 2932, 2860, 1633 (C=O), 1515, 1455, 1381, 1315, 1237, 1132, 986, and 958 cm^{-1}; UV (MeOH): 209, 276 and 316 nm; ^1H NMR ($CDCl_3$): d 0.89 (3H, t, *J* = 8.5 Hz, C-5'H), 1.20 (3H, d, *J* = 6.8 Hz, $CHCH_3$), 1.26-1.32 (4H, m, C-3'H and C-4'H), 1.37-1.49 (1H, m, C-2'H_a), 1.75-1.83 (1H, m, C-2'H_b), 3.39 (1H, m, C-1'H), 6.37-6.41 (2H, m, C-3H and C-5H), 7.68 (1H, d, *J* = 9.4 Hz, C-6H) and 13.13 (1H, s, chelated OH); ^{13}C NMR ($CDCl_3$): d 13.91 (C-5'), 17.61 ($CHCH_3$), 22.55, 29.62, and 33.74 (C-2', C-3', and C-4'), 39.79 (C-1'), 103.75 (C-3), 107.74 (C-5), 113.22 (C-1), 132.13 (C-6), 162.71, and 165.79 (C-2 and C-4) and 209.27 (C=O); EIMS, *m/z* (% rel. int.): 222 ($[M]^+$, 20), 163(40), 137(95), 105(10), 81(25), 69(15) and 44(100).

General Procedure of Acetylation of (±)-2,4-Dihydroxyphenyl Alkyl Ketones 5-8: Preparation of (±)-Diacetates 9-12

(±)-2,4-Dihydroxyphenyl alkyl ketone (**5-8**, 3 mmol) was stirred with acetic anhydride and catalytic amount of *N,N*-dimethylaminopyridine (DMAP) at 22-25°C. The reaction was monitored periodically by TLC and on completion, poured into ice-cold water (50 ml). The compound thus obtained was extracted with dichloromethane (2 × 25 ml), the combined organic layer dried over anhydrous sodium sulphate and solvent removed under reduced pressure and the product purified by column chromatography using petroleum ether-ethyl acetate as eluent.

(±)-2,4-Diacetoxyphenyl (1-Phenyl)ethyl Ketone (9)

It was obtained as an oil (0.93 g) in 95% yield. R_f: 0.45; IR (nujol): 2967, 1772 ($OCOCH_3$), 1686(C=O), 1637, 1607, 1582, 1492, 1454, 1416, 1369, 1303, 1197, 1149, 1122, 1014, 906 and 819 cm^{-1}; UV (MeOH): 258 and 320 nm; ^1H NMR ($CDCl_3$): d 1.47 (3H, d, *J* = 6.8 Hz, C-2'H), 2.25 and 2.30(6H, 2s, 3H each, 2 x $OCOCH_3$), 4.47 (1H, q, *J* = 6.8 Hz, C-1'H), 6.90 (1H, d, *J* = 2.1 Hz, C-3H), 6.96 (1H, dd, *J* = 8.6 and 2.1 Hz, C-5H), 7.20-7.31 (5H, m, aromatic protons) and 7.65 (1H, d, *J* = 8.6 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 20.78(C-2'), 22.05 and 22.56 (2 × $OCOCH_3$), 51.99 (C-1'), 118.12 (C-1), 119.96 and 120.46 (C-3 and C-5), 128.58, 129.25 and 130.04 (C-2", C-3", C-4", C-5" and C-6"), 132.30 (C-6), 134.85 (C-1"), 151.52 and 154.85 (C-2 and C-4), 169.77 and 170.54 (2 × $COCH_3$) and 200.98 (C=O); EIMS, *m/z* (% rel. int.): 325 ($[M-1]^+$, 2.5), 255(35), 221(35), 207(2), 179(55), 165(5), 137(100), 119(5), 91(20), 81(5), 58(22), and 43(95).

(±)-2,4-Diacetoxyphenyl (1-Phenyl)propyl Ketone (10)

It was obtained as an oil (0.92 g) in 90% yield. R_f: 0.45; IR (nujol): 2967, 1770, 1683, 1607, 1582, 1494, 1454, 1416, 1369, 1196, 1149, 1122, 1014, 906, and 820 cm^{-1}; UV (MeOH): 281 and 316 nm; ^1H NMR ($CDCl_3$): d 0.88 (3H, t, J = 7.3 Hz, C-3'H), 1.75-1.84 (1H, m, C-2'H_a), 2.10-2.21 (1H, m, C-2'H_b), 2.26 and 2.30(6H, 2s, 3H each, 2 × $OCOCH_3$), 4.22 (1H, t, J = 7.3 Hz, C-1'H), 6.90 (1H, d, J = 2.1 Hz, C-3H), 6.98 (1H, dd, J = 8.5 and 2.1 Hz, C-5H), 7.19-7.33 (5H, m, aromatic protons) and 7.65 (1H, d, J = 8.5 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 12.57 (C-3'), 21.33 and 21.46 (2 × $OCOCH_3$), 27.47 (C-2'), 58.74 (C-1'), 115.50 (C-1), 117.77 (C-3), 119.20 (C-5), 127.54, 128.99, and 129.26 (C-2", C-3", C-4", C-5", and C-6"), 131.01 (C-6), 139.25 (C-1"), 150.24, and 153.65 (C-2 and C-4), 168.72, and 169.46 (2 × $COCH_3$) and 199.79 (C=O); EIMS, *m/z* (% rel. int.): 340 ($[M]^+$, 5), 298(15), 256(10), 221(95), 179(95), 137(100), 91(95), 77(15), 65(10) and 43(95).

(±)-2,4-Diacetoxyphenyl (1-Methyl)propyl Ketone (11)

It was obtained as an oil (0.75 g) in 90% yield. R_f: 0.50; IR (nujol): 2928, 1771 ($OCOCH_3$), 1685 (C=O), 1609, 1457, 1370, 1197, 1014, and 905 cm^{-1}; UV (MeOH): 218 and 259 nm; ^1H NMR ($CDCl_3$): d 0.87 (3H, t, J = 7.3 Hz, C-3'H), 1.13 (3H, d, J = 6.8 Hz, $CHCH_3$), 1.37-1.47 (1H, m, C-2'H_a), 1.72-1.82 (1H, m, C-2'H_b), 2.28 and 2.30 (6H, 2s, 3H each, 2 × $OCOCH_3$), 3.18 (1H, m, C-1'H), 6.96 (1H, d, J = 2.2 Hz, C-3H), 7.09 (1H, dd, J = 8.5 and 2.2 Hz, C-5H) and 7.73 (1H, d, J = 8.5 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 11.96 (C-3'), 16.53 ($CHCH_3$), 21.30 and 21.41 (2 × $OCOCH_3$), 26.61 (C-2'), 45.41 (C-1'), 117.88 (C-3), 119.38 (C-5), 128.94 (C-1), 130.76 (C-6), 150.33, and 153.78 (C-2 and C-4), 168.74 and 169.34 (2 ´ $COCH_3$) and 203.80 (C=O); EIMS, *m/z* (% rel. int.): 278 ($[M]^+$, 5), 269(5), 227(5), 174(15), 137(20), 95(20), 69(25), 57(35) and 44(100).

(±)-2,4-Diacetoxyphenyl (1-Methyl)pentyl Ketone (12)

It was obtained as an oil (0.83 g) in 90% yield. R_f: 0.50; IR (nujol): 2932, 2859, 1770 ($OCOCH_3$), 1683 (C=O), 1608, 1581, 1494, 1462, 1415, 1370, 1196, 1148, 1114, 1044, 1014, 982, and 959 cm^{-1}; UV (MeOH): 245 nm; ^1H NMR ($CDCl_3$): d 0.87 (3H, t, J = 6.5 Hz, C-5'H), 1.22-1.28 (5H, m, C-2'H_a, C-3'H and C-4'H), 1.25 (3H, d, J = 9.1 Hz, $CHCH_3$), 1.58-1.59 (1H, m, C-2'H_b), 2.28 and 2.32 (6H, 2s, 3H each, 2 × $OCOCH_3$), 3.23(1H, m, C-1'H), 6.96 (1H, d, J = 2.2 Hz, C-3H), 7.09 (1H, dd, J = 8.6 and 2.2 Hz, C-5H) and 7.74 (1H, d, J = 8.6 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 14.31 (C-5'), 17.07 ($CHCH_3$), 21.37 and 21.50 (2 ´ $OCOCH_3$), 22.95, 29.89, 33.67 (C-2', C-3' and C-4'), 44.00 (C-1'), 117.92 (C-3), 119.40 (C-5), 130.79 (C-1), 132.17 (C-6), 150.39 and 153.80 (C-2 and C-4),

168.80 and 169.42 (2 × *C*OCH$_3$) and 203.98 (C=O); EIMS, *m/z* (% rel. int.): 266(45), 251(20), 221(90), 179(95), 137(100), 110(10), 97(75), 85(15), 57(25) and 43(95).

Deacetylation of 2,4-Diacetoxyphenyl Methyl Ketone Mediated by PPL Pre-incubated with Phenyl Methyl Ketone

To a solution of phenyl methyl ketone (0.24 g, 2 mmol) in dry THF (30 ml), PPL (250 mg) was added and the suspension was stirred at 40-42°C for 1 hour in an incubator shaker. A solution of 2,4-diacetoxyphenyl methyl ketone (0.47 g, 2 mmol) in THF (10 ml) was added into the incubated suspension, followed by the addition of *n*-butanol (5 molar equivalent) and stirring continued at the same temperature for 24 hours. The reaction was quenched by filtering off the enzyme, solvent was removed under reduced pressure and the crude product was purified by column chromatography to afford completely deacetylated 2,4-dihydroxyphenyl methyl ketone in 75% yield (m.p. 140-142°C, lit. [20] m.p. 145-147°C), together with phenyl methyl ketone.

Deacetylation of 2,4-Diacetoxyphenyl (1-Phenyl)propyl Ketone (10) Mediated by PPL Pre-incubated with Phenyl Methyl Ketone

To a solution of phenyl methyl ketone (0.24 g, 2 mmol) in dry THF (30 ml), PPL (250 mg) was added and the suspension was stirred at 40-42°C for 1 hour in an incubator shaker. A solution of 2,4-diacetoxyphenyl (1-phenyl)propyl ketone (0.68 g, 2 mmol) in THF (10 ml) was added into the incubated suspension, followed by the addition of *n*-butanol (5 molar equivalent) and stirring continued at the same temperature for 48 hours. The reaction was quenched by filtering off the enzyme, solvent was removed under reduced pressure and the crude product purified by column chromatography to afford the completely deacetylated compound (+)-2,4-dihydroxyphenyl (1-phenyl)propyl ketone (**6**) as a viscous oil in 33.7% yield, $[a]_D^{25}$ +25.0 (*c* 0.96 in $CHCl_3$) together with a partially deacetylated compound (-)-2-acetoxy-4-hydroxyphenyl (1-phenyl)propyl ketone (**14**) as a viscous oil in 14% yield, R_f: 0.35; $[a]D^{25}$ -69.56 (*c* 0.46 in $CHCl_3$); IR (nujol): 3361, 2966, 2931, 2875, 1737 (OCOCH$_3$), 1673(C=O), 1611, 1501, 1450, 1368, 1312, 1233, 1162, 1124, 1016, 973, 903, and 852 cm^{-1}; UV (MeOH): 276 nm; ^{1}H NMR ($CDCl_3$): d 0.86(3H, t, *J* = 7.3 Hz, C-3'H), 1.69-1.83(1H, m, C-2'H$_a$), 2.04-2.16 (1H, m, C-2'H$_b$) 2.33 (3H, s, OCOCH$_3$), 4.22 (1H, t, *J* = 7.2 Hz, C-1'H), 6.39 (1H, d, *J* = 2.3 Hz, C-3H), 6.44 (1H, dd, *J* = 8.5 Hz and 2.3 Hz, C-5H), 7.11-7.30 (5H, m, aromatic protons) and 7.55 (1H, d, *J* = 8.5 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 12.21 (C-3'), 21.17 (OCO*C*H$_3$), 27.10 (C-2'), 57.28 (C-1'), 111.31 (C-3), 113.01 (C-5), 122.83 (C-1), 126.95, 128.14, and 128.79 (C-2", C-3", C-4", C-5", and C-6"), 132.19 (C-6), 139.59 (C-1"), 151.17 (C-4), 160.30 (C-2), 170.49

(*C*OCH$_3$) and 198.68 (C=O); EIMS, *m/z* (% rel. int.): 298 ([M]$^+$, 5), 256(5), 221(20), 179(42), 165(2), 137(100), 119(5), 91(25), 81(10), 57(5) and 44(12).

General Procedure of Enzymatic Deacetylation of (±)-2,4-Diacetoxyphenyl Alkyl Ketones (9-12)

To a solution of the (±)-2,4-diacetoxyphenyl alkyl ketone (**9-12**, 2 mmol) in anhydrous tetrahydrofuran (20-25 ml), *n*-butanol (5 equiv.) was added, followed by the addition of unmodified porcine pancreatic lipase (200-300 mg). The suspension was incubated in a shaker at 40-42°C and progress of the reaction was monitored periodically by HPLC and/or TLC examination. After about 50% conversion of the starting material into the product, the reaction mixture was quenched by filtering off the enzyme and the solvent removed under reduced pressure to afford a thick oil which was purified by column chromatography over silica gel using a gradient solvent system of petroleum ether:ethyl acetate to afford optically enriched, unreacted (+)-2,4-diacetoxyphenyl alkyl ketones **9-12** and the enzymatically partially deacetylated (-)-2-acetoxy-4-hydroxyphenyl alkyl ketones **13-16** in 60 to 81% and 54 to 72% yields [23], respectively (Table 2). All the monoacetates **13-16** were found to be new compounds and characterized on the basis of their spectral analysis. The spectral data of unreacted (+)-2,4-diacetoxyphenyl alkyl ketones **9-12** were found to be identical with the data of (±)-2,4-diacetoxyphenyl alkyl ketones **9-12** synthesized by peracetylation of (±)-2,4-dihydroxyphenyl alkyl ketones **5-8**.

(-)-2-Acetoxy-4-hydroxyphenyl (1-Phenyl)ethyl Ketone (13)

It was obtained as a white amorphous solid (0.15 g) in 55% yield, m.p. 135-137°C. R_f: 0.35; $[a]^{25}_D$ -33.0 (*c* 3.33, $CHCl_3$); IR (nujol): 3348, 2969, 2934, 2877, 1738 ($OCOCH_3$), 1631 (C=O), 1505, 1451, 1374, 1313, 1230, 1131, 1014, 985, and 952 cm^{-1}; UV (MeOH): 277 and 322 nm; ^{1}H NMR ($CDCl_3$): d 1.43(3H, d, *J* = 6.8 Hz, C-2'H), 2.35(3H, s, $OCOCH_3$), 4.50 (1H, q, *J* = 6.8 Hz, C-1'H), 6.40-6.45 (2H, m, C-3H and C-5H), 7.16-7.32 (5H, m, aromatic protons) and 7.55 (1H, d, *J* = 8.2 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 19.81(C-2'), 21.60 ($OCOCH_3$), 49.86(C-1'), 110.62 and 111.75 (C-3 and C-5), 122.35 (C-1), 127.27, 127.59, and 132.84 (C-2", C-3", C-4", C-5", and C-6"), 133.25 (C-6), 141.89 (C-1"), 151.78 (C-4), 161.00 (C-2), 170.98 (*C*OCH$_3$) and 199.29 (C=O); EIMS, *m/z* (% rel. int.): 284 ([M]$^+$, 5), 221(5), 179(20), 137(100), 105(10), 81(5), and 44(85).

(-)-2-Acetoxy-4-hydroxyphenyl (1-Phenyl)propyl Ketone (14)

It was obtained as a viscous oil (0.17 g) in 60% yield. R_f: 0.35; $[a]^{25}_D$ - 50.8 (*c* 1.70, $CHCl_3$). IR, UV, ^{1}H NMR, ^{13}C NMR, and EI mass spectral data were

found to be identical with the corresponding data of partial acetate (-)-**14** obtained by deacetylation of (±)-**10** mediated by PPL pre-incubated with phenyl methyl ketone.

(-)-2-Acetoxy-4-hydroxyphenyl (1-Methyl)propyl Ketone (15)

It was obtained as an oil (0.17 g) in 72% yield. R_f: 0.40; $[a]^{25}_D$ - 41.4 (*c* 1.83, $CHCl_3$); IR (nujol): 3346, 2959, 2932, 2860, 1771, 1738 ($OCOCH_3$), 1614 (C=O), 1504, 1455, 1371, 1313, 1238, 1160, 1120, 1044, 1015, 985, 958, 899, and 851 cm^{-1}; UV (MeOH): 273 and 316 nm; ^1H NMR ($CDCl_3$): d 0.89 (3H, t, *J* = 7.3 Hz, C-3'H), 1.13 (3H, d, *J* = 6.8 Hz, $CHCH_3$), 1.37-1.44 (1H, m, C-2'H_a), 1.71-1.80 (1H, m, C-2'H_b), 2.34 (3H, s, $OCOCH_3$), 3.20 (1H, m, C-1'H), 6.38 (1H, br s, OH), 6.52 (1H, d, *J* = 2.4 Hz, C-3H), 6.70 (1H, dd, *J* = 8.6 and 2.4 Hz, C-5H) and 7.68(1H, d, *J* = 8.6 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 12.08 (C-3'), 16.89 ($CHCH_3$), 21.53 ($OCOCH_3$), 26.96 (C-2'), 44.62 (C-1'), 111.77 (C-3), 113.47 (C-5), 123.46 (C-1), 132.23 (C-6), 151.64 (C-4), 160.42 (C-2), 170.37 ($COCH_3$) and 203.42 (C=O); EIMS, *m/z* (% rel. int.): 218 ($[M-18]^+$, 5), 174(20), 123(18), 109(25), 81(30), 69(45), 57(50) and 44(100).

(-)-2-Acetoxy-4-hydroxyphenyl (1-Methyl)pentyl Ketone (16)

It was obtained as an oil (0.14g) in 54% yield. R_f: 0.40; $[a]^{25}_D$ - 41.6 (*c* 2.16, $CHCl_3$); IR (nujol): 3345, 2959, 2932, 2860, 1771, 1738 ($OCOCH_3$), 1614 (C=O), 1504, 1455, 1371, 1313, 1238, 1160, 1120, 1044, 1015, 985, and 958 cm^{-1}; UV (MeOH): 272 and 315 nm; ^1H NMR ($CDCl_3$): d 0.86 (3H, t, *J* = 6.8 Hz, C-5'H), 1.12 (3H, d, *J* = 6.8 Hz, $CHCH_3$), 1.25-1.39 (5H, m, C-2'H_a, C-3'H and C-4'H), 1.71-1.75 (1H, m, C-2'H_b), 2.34 (3H, s, $OCOCH_3$), 3.26 (1H, m, C-1'H), 6.49 (1H, d, *J* = 2.4 Hz, C-3H), 6.67 (1H, dd, *J* = 8.6 and 2.4 Hz, C-5H) and 7.67 (1H, d, *J* = 8.6 Hz, C-6H); ^{13}C NMR ($CDCl_3$): d 14.30 (C-5'), 17.35 ($CHCH_3$), 21.54 ($OCOCH_3$), 23.12, 29.89, 30.06 (C-2', C-3' and C-4'), 42.99 (C-1'), 111.80 (C-3), 113.62 (C-5), 122.93 (C-1), 132.22 (C-6), 151.57 (C-4), 160.94 (C-2), 170.82 ($COCH_3$) and 203.92 (C=O); EIMS, *m/z* (% rel. int.): 264 ($[M]^+$, 5), 235(35), 221(40), 207(15), 179(35), 151(20), 137(100), 109(20), 81(20), 69(20) and 43(95).

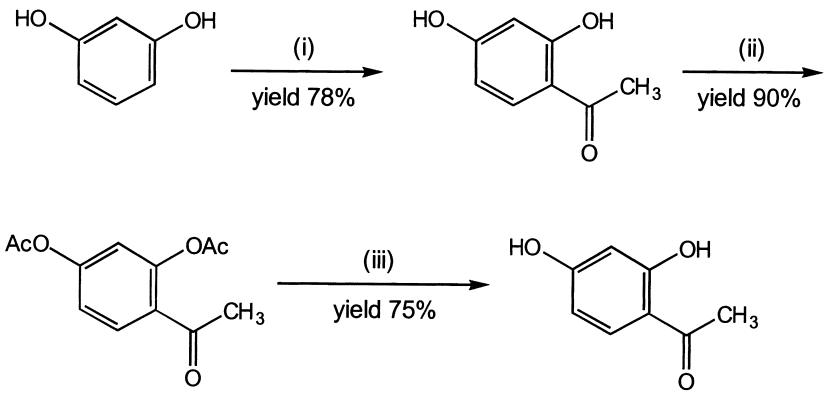
General Procedure of Chemical Acetylation of (-)-2-Acetoxy-4-hydroxyphenyl Alkyl Ketones 13-16

(-)-2-Acetoxy-4-hydroxyphenyl alkyl ketone (**13-16**, 50-60 mg) was stirred with acetic anhydride and catalytic amount of DMAP for 12-15 hours at 22-25°C. On completion of acetylation, the reaction mixture was poured into ice-cold water (20 ml) and the compound extracted with dichloromethane (2 ´ 10 ml) and puri-

fied by preparative TLC to afford the pure (-)-2,4-diacetoxyphenyl alkyl ketones **9-12** in quantitative yields. The spectral data of (-)-diacetates **9-12** were found to be identical with the data of (±)-diacetates **9-12**.

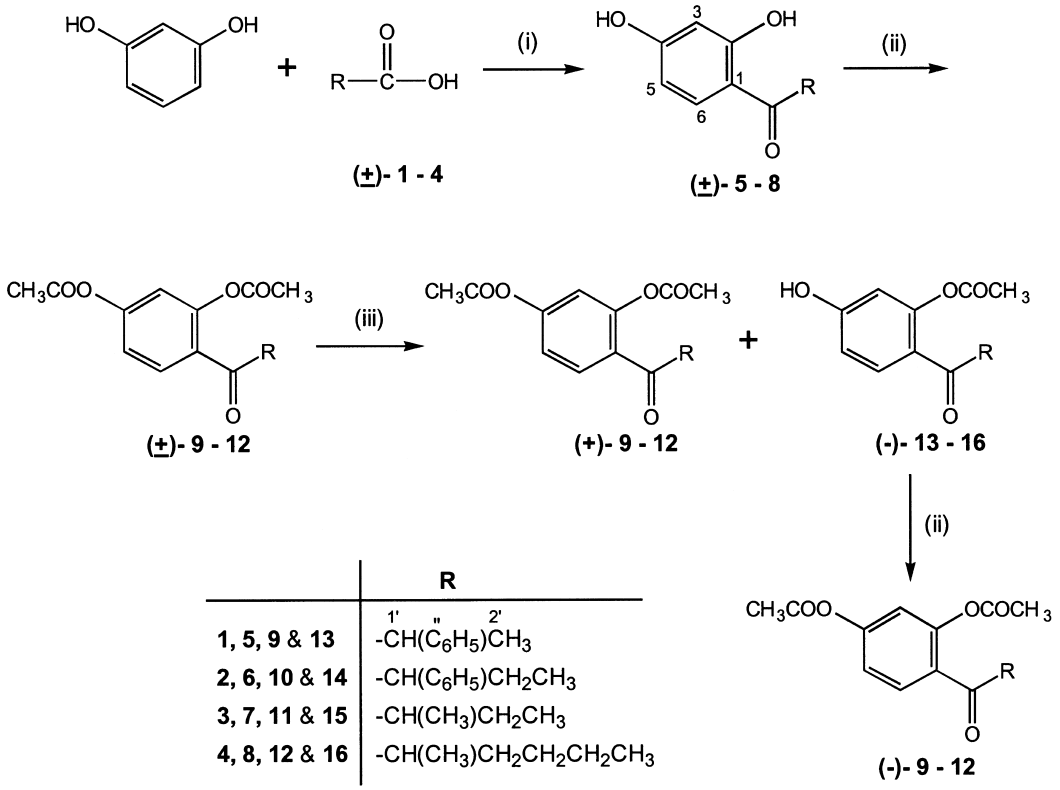
RESULTS AND DISCUSSION

2,4-Dihydroxyphenyl methyl ketone was synthesized by a known procedure [20] and acetylated by acetic anhydride-pyridine method to yield 2,4-diacetoxyphenyl methyl ketone in quantitative yield (Scheme 1) [21]. The racemic aryl alkyl ketones, *i.e.,* (±)-2,4-dihydroxyphenyl (1-phenyl)ethyl ketone (**5**), (±)-2,4-dihydroxyphenyl (1-phenyl)propyl ketone (**6**), (±)-2,4-dihydroxyphenyl (1-methyl)propyl ketone (**7**) and (±)-2,4-dihydroxyphenyl (1-methyl)pentyl ketone (**8**) were prepared by Nencki reaction [22] of resorcinol with the corresponding racemic aliphatic acids, *i.e.,* (±)-2-phenylpropanoic acid (**1**), (±)-2-phenylbutanoic acid (**2**), (±)-2-methylbutanoic acid (**3**) and (±)-2-methylhexanoic acid (**4**), respectively in the presence of fused $ZnCl_2$ at 150°C in 50 to 55% yields (Scheme 2). The diacetates of dihydroxy compounds **5-8**, *i.e.,* (±)-2,4-diacetoxyphenyl (1-phenyl)ethyl ketone (**9**), (±)-2,4-diacetoxyphenyl (1-phenyl)propyl ketone (**10**), (±)-2,4-diacetoxyphenyl (1-methyl)propyl ketone (**11**) and (±)-2,4-diacetoxyphenyl (1-methyl)pentyl ketone (**12**) were prepared by acetylation using the acetic anhydride/DMAP method in quantitative yields. All the (±)-dihydroxyphenyl alkyl ketones **5-8** and (±)-diacetoxyphenyl alkyl ketones **9-12** were found to be new compounds in the literature and have been characterized successfully by us on the basis of their spectral data (IR, UV, ^{1}H NMR, ^{13}C NMR and EIMS).



Reagents and Conditions : (i) acetic acid, fused $ZnCl_2$, 150°C; (ii) acetic anhydride, pyridine, 28°C; (iii) PPL pre-incubated with phenyl methyl ketone, THF, *n*-butanol (5 equivalents), 40-42 °C

Scheme 1.



	R
1, 5, 9 & 13	-CH(C6H5)CH3
2, 6, 10 & 14	-CH(C6H5)CH2CH3
3, 7, 11 & 15	-CH(CH3)CH2CH3
4, 8, 12 & 16	-CH(CH3)CH2CH2CH2CH3

Reagents and Conditions : (i) fused $ZnCl_2$, 150°C; (ii) acetic anhydride, DMAP; (iii) PPL, THF, *n*-butanol (5 equivalents), 40-42 °C.

Scheme 2.

Phenyl methyl ketone was incubated with PPL in THF at 40-42°C in an incubator shaker, and this PPL-ketone system was used to catalyze the deacetylation of 2,4-diacetoxyphenyl methyl ketone leading to the formation of completely deacetylated 2,4-dihydroxyphenyl methyl ketone in 75% yield (Scheme 1). The formation of dihydroxy ketone indicates that PPL, which in its unmodified form exclusively mediates the deacetylation of *para*-acetoxy function of 2,4-diacetoxyphenyl alkyl ketone over the *ortho*-acetoxy group with respect to carbonyl function [13], does not show any preference for the *ortho* or *para* acetoxy function when it is preincubated with phenyl methyl ketone. This observation clearly confirms our proposed mechanism of action of PPL in THF involving a dynamic Schiff's base complex formation between the x-amino group of the lysine residue in the active site of PPL and the keto group directly attached to the benzenoid ring of the substrate. In the modified PPL, obtained by preincubation with phenyl

methyl ketone, the x-amino group of its lysine residue is already engaged in the Schiff's base formation with the carbonyl group of this ketone, the thus formed PPL-phenyl methyl ketone system fails to discriminate between the *ortho* and *para* acetoxy functions of 2,4-diacetoxyphenyl alkyl ketone leading to the formation of the completely deacetylated dihydroxyphenyl alkyl ketone. Similarly racemic diacetoxy ketone (±)-2,4-diacetoxyphenyl (1-phenyl)propyl ketone (**10**) was incubated with PPL-phenyl methyl ketone system leading to the formation of a completely deacetylated compound (+)-2,4-dihydroxyphenyl (1-phenyl)propyl ketone (**6**) and a partially deacetylated compound (-)-2-acetoxy-4-hydroxyphenyl (1-phenyl)propyl ketone (**14**). This random selectivity of the modified PPL (obtained by pre-incubation with phenyl methyl ketone) is also in conformity with our hypothesis on the mechanism of action of PPL in THF [17]. The dihydroxy and monohydroxy products formed during the deacetylation of (±)-**10** catalyzed by PPL were found to be optically active which revealed that the rates of deacetylation of the acetoxy functions of the two enantiomers in the racemic mixture are unequal.

In order to investigate the possibility of enantiomeric resolution through the selective deacetylation of the acetoxy function present in the phenyl ring of racemic aryl alkyl ketones in general, (±)-2,4-diacetoxyphenyl (1-phenyl)ethyl ketone (**9**) and (±)-2,4-diacetoxyphenyl (1-phenyl)propyl ketone (**10**) were incubated with unmodified PPL in THF and the reaction was stopped by filtering off the enzyme after about 50% conversion of the starting diacetate to a slow moving product on TLC. It was found that the enzyme selectively deacetylates the *para* acetoxy function over the *ortho* acetoxy function with respect to the nuclear carbonyl group of diacetoxyphenyl alkyl ketones **9** and **10** leading to the formation of 2-acetoxy-4-hydroxyphenyl (1-phenyl)ethyl ketone (**13**) and 2-acetoxy-4-hydroxyphenyl (1-phenyl)propyl ketone (**14**) in 55 and 60% yields [23], respectively (Table 1). In addition to the regioselectivity in deacetylation of (±)-2,4-diacetoxyphenyl alkyl ketones **9** and **10**, the enzyme also showed enantioselectivity and preferentially deacetylated the *para* acetoxy function of one enantiomer over the *para* acetoxy function of the other leading to the formation of optically active (-)-2-acetoxy-4-hydroxyphenyl (1-phenyl)ethyl ketone (**13**) and (-)-2-acetoxy-4-hydroxyphenyl (1-phenyl)propyl ketone (**14**) (Table 2).

In addition to the selective deacetylation study on diacetoxyphenyl alkyl ketones **9** and **10** having phenyl substituents on alkyl moiety, ketones with methyl substituents, *i.e.,* (±)-2,4-diacetoxyphenyl (1-methyl)propyl ketone (**11**) and (±)-2,4-diacetoxyphenyl (1-methyl)pentyl ketone (**12**) were also incubated with unmodified PPL in THF until about 50% conversion of the starting material into the products to investigate the effect of nature of different alkyl moieties on regio- and enantioselective capabilities of the enzyme. It was observed that the enzyme deacetylates the *para* acetoxy group of compounds **11** and **12** exclusively over the *ortho* acetoxy group as in the case of compounds **9** and **10** leading to the formation of (-)-2-acetoxy-4-hydroxyphenyl (1-methyl)propyl ketone (**15**) and (-)-2-acetoxy-4-hydroxyphenyl (1-methyl)pentyl ketone (**16**) in 72 and 54% yield [23],

Table 1. Selective Deacetylation of (±)-2,4-Diacetoxyphenyl Alkyl Ketones Mediated by PPL in THF at 40–42°C in the Presence of *n*-Butanol[a,b]

Substrate	Time(h)	Products: deacetylated (−)-monoacetates and recovered, unreacted (+)-diacetates	(% yield)[23]
(±)-2,4-Diacetoxyphenyl (1-phenyl)ethyl ketone (**9**)	12	(−)-2-Acetoxy-4-hydroxyphenyl (1-phenyl)ethyl ketone (**13**)	55
		and	
		(+)-2,4-Diacetoxyphenyl (1-phenyl)ethyl ketone (**9**)	63
(±)-2,4-Diacetoxyphenyl (1-phenyl)propyl ketone (**10**)	12	(−)-2-Acetoxy-4-hydroxyphenyl (1-phenyl)propyl ketone (**14**)	60
		and	
		(+)-2,4-Diacetoxyphenyl (1-phenyl)propyl ketone (**10**)	75
(±)-2,4-Diacetoxyphenyl (1-methyl)propyl ketone (**11**)	12	(−)-2-Acetoxy-4-hydroxyphenyl (1-methyl)propyl ketone (**15**)	72
		and	
		(+)-2,4-Diacetoxyphenyl (1-methyl)propyl ketone (**11**)	81
(±)-2,4-Diacetoxyphenyl (1-methyl)pentyl ketone (**12**)	10	(−)-2-Acetoxy-4-hydroxyphenyl (1-methyl)pentyl ketone (**16**)	54
		and	
		(+)-2,4-Diacetoxyphenyl (1-methyl)pentyl ketone (**12**)	60

[a]All these reactions, when performed under identical conditions but without adding porcine pancreatic lipase, did not yield any product.

[b]All deacetylation reactions were stopped by filtering off the enzyme after about 50% conversion of the starting racemic diacetates to the product, i.e., monoacetates.

Table 2. Optical Rotation Values of PPL-Catalyzed Deacetylation Products (−)-**13-16**; the Recovered, Unreacted Diacetates (+)-**9-12** and the Compounds (−)-**9-12** Obtained by Chemical Acetylation of (−)-**13-16**

	$[\alpha]^{25}_{D}$ Values in chloroform		
Substrate (racemic)	Monoacetates **13–16**	Recovered diacetates **9–12**	Diacetates **9–12** prepared by chemical acetylation of monoacetates **13–16**
9	**13**: (−) 33.0	**9**: (+) 26.1	**9**: (−) 18.0
10	**14**: (−) 50.8	**10**: (+) 30.0	**10**: (−) 21.8
11	**15**: (−) 41.4	**11**: (+) 32.5	**11**: (−) 23.9
12	**16**: (−) 41.6	**12**: (+) 37.9	**12**: (−) 41.5

respectively (Table 1). Again there was a kinetic resolution during the enzyme-catalyzed deacetylation of (±)-**11** and (±)-**12** leading to the formation of *leavorotatory* 2-acetoxy-4-hydroxyphenyl alkyl ketones **15** and **16** (Table 2). The time required for about 50% conversion of 2,4-diacetoxyphenyl alkyl ketones **9-12** to 2-acetoxy-4-hydroxyphenyl alkyl ketones **13-16** in the presence of PPL is almost the same (Table 1), thus indicating that the nature of the moiety attached to the alkyl residue of the diacetoxyphenyl alkyl ketone does not affect the selectivity of the enzymatic reaction, *i.e.* the enzyme and alkyl group interaction does not play any crucial role in the de-esterification of phenolic acetoxy function. These results are in accordance with our earlier proposed hypothesis on the mechanism of action of PPL in THF involving a dynamic Schiff's base complex formation between the x-amino group of the lysine residue present in the active site of PPL and the keto group directly attached to the benzenoid ring [17].

The products of enzymatic deacetylation reactions, *i.e.*, 2-acetoxy-4-hydroxyphenyl alkyl ketones **13-16** are new compounds and have been fully characterized on the basis of their spectral data (IR, UV, ^{1}H NMR, ^{13}C NMR, and EIMS). The presence of hydroxyl group at the *para* position with respect to the nuclear carbonyl group in the compounds **13-16** was supported by the absence of chelated hydroxyl group in them and the presence of only one acetoxy function in their ^{1}H NMR spectra and by the nature of their color reaction with 10% alcoholic $FeCl_3$ solution on TLC. This result was further supported by ^{13}C NMR and other spectral data. All these reactions, when performed under identical conditions but without addition of the enzyme, did not yield any product.

In order to determine the enantiomeric excess (*ee*) values of enzymatically deacetylated (-)-2-acetoxy-4-hydroxyphenyl alkyl ketones **13-16**, the separation of two enantiomers of corresponding (±)-2,4-diacetoxyphenyl alkyl ketones **9-12** was tried by HPLC using chiracel OJ and chiracel OD chiral columns; separation of enantiomers was not observed. The enantiomeric excess determination tried by chiral shift ^{1}H NMR spectroscopic technique using (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(+)-TFAE] shift reagent also failed as separation of the signals of diastereoisomeric complexes between ketone and chiral shift reagent was not observed in their ^{1}H NMR spectra. Thus enantiomeric excess of (-)-2-acetoxy-4-hydroxyphenyl alkyl ketones **13-16** could not be determined. However, to show that the lipase exhibits enantioselectivity and yields optically enriched (-)-monohydroxy ketones **13-16**, these ketones were acetylated by acetic anhydride/DMAP method to give the corresponding diacetoxy ketones **9-12,** and their optical rotation values were measured. The comparison of optical rotation values of the recovered, unreacted (+)-2,4-diacetoxyphenyl alkyl ketones **9-12** with the corresponding (-)-2,4-diacetoxyphenyl alkyl ketones **9-12** obtained by chemical acetylation of (-)-monoacetates **13-16** revealed that they are comparable and had opposite signs of rotation (Table 2). This indicates that the optical enrichment during enzymatic deacetylation is of high order. It may be mentioned that during the course of this enzyme-assisted selective deacetylation studies, 12 new compounds, *i.e.*, **5-16** have been obtained, eight of them in their optically active forms.

CONCLUSION

The present study confirms the hypothesis of Schiff's base formation during deacetylation on peracetylated polyphenolic aromatic ketones catalyzed by PPL in THF. The regioselective capability of PPL has successfully been used for the enantiomeric separation of racemic 2,4-diacetoxyphenyl alkyl ketones **9-12**, which are precursors of analogs of a potent antifungal coumarin, 7-acetoxy-4-(1-ethyl)pentyl-3-phenyl-2*H*-1-benzopyran-2-one [5]. Thus, the optically enriched phenyl alkyl ketones could be used for the synthesis of analogs of such optically pure antifungal coumarins so that the activity of both the enantiomers could be compared and compounds with better activity can be selected. As it is difficult to synthesize such compounds in enantiomerically enriched forms by purely chemical methods, the biocatalytic approach reported herein may find utility in the synthesis of optically enriched compounds of this class.

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23. Yields were calculated by assuming corresponding single enantiomer as 100% in the starting (±)-2,4-diacetoxyphenyl alkyl ketones **9-12**.