Lipase-catalysed Selective Deacetylation of Peracetylated Benzopyranones†

Virinder S. Parmar,* Ashok K. Prasad, Nawal K. Sharma, Anand Vardhan, Hari N. Pati, Sunil K. Sharma and Kirpal S. Bisht

Department of Chemistry, University of Delhi, Delhi-110 007, India

Molecular recognition has been observed in the hydrolysis of peracetates of benzopyranones by lipases from porcine pancreas and *Candida cylindracea* in organic solvents; the acetoxy group(s) at positions other than *peri* or *ortho* to the carbonyl group are hydrolysed preferentially.

Benzopyranone derivatives occur widely in nature and many of their analogues possess a variety of biological activities, *i.e.* antitumour, antiviral, antibiotic and antifungal. Selective protection/deprotection steps are often employed for the synthesis of compounds of this class,[1,2] which increase the number of steps for their total synthesis and final yields are quite low.[3,4] Numerous work has been published on enzyme-assisted regioselective deacylation of aliphatic alcohols,[5–7] however, only one paper has appeared reporting the regioselective deacylation of polyphenols.[8]

Aiming to simplify the total synthesis of such compounds, we have systematically investigated the deacetylation of different peracetylated model compounds‡ representing four

† Part of this work has been presented at the IUPAC-NOST International Symposium on Enzymes in Organic synthesis held in New Delhi, India on 6–9 January, 1992.

‡ The peracetylated derivatives **1–5** were prepared by acetylation of the corresponding polyphenolic compounds by acetic anhydride–pyridine method either at room temp. (32 °C) or at 100 °C. The earlier known acetates were identified by comparison of their spectral data with those reported in the literature, while the new acetylated compounds were unambiguously identified on the basis of their ^{1}H NMR and IR spectral data.

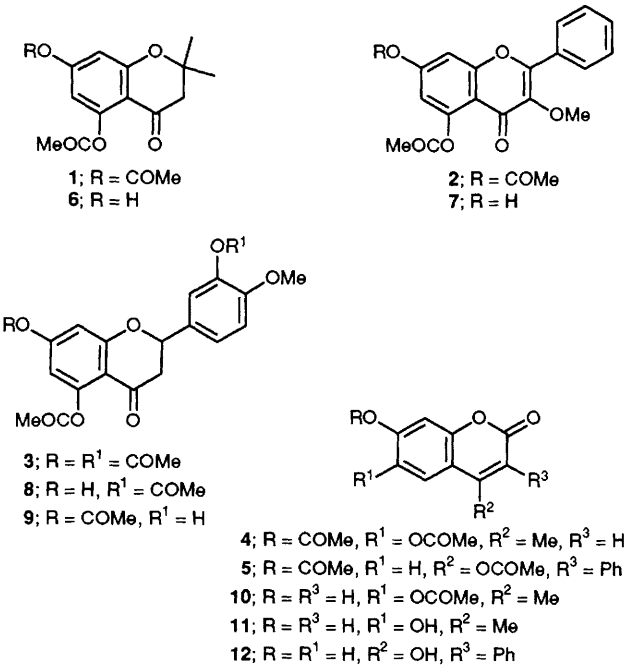
Table 1

Substrate	Reaction conditions (T = 42–45 °C)	Product [yield (%)]
1	THF, PPL	5-Acetoxy-7-hydroxy-2,2-dimethylchromanone **6**[a] [73]
2	DIPE, CCL	5-Acetoxy-7-hydroxy-3-methoxyflavone **7**[a] [65]
3	THF, PPL	5,3′-Diacetoxy-7-hydroxy-4′-methoxyflavanone **8**[a] [55]; 5,7-diacetoxy-3′-hydroxy-4′-methoxyflavanone **9**[a] [23]
4	THF, PPL	6-Acetoxy-7-hydroxy-4-methylcoumarin **10**[a] [65]; 6,7-dihydroxy-4-methylcoumarin **11**[b] [15]
5	THF, PPL	4,7-Dihydroxy-3-phenylcoumarin **12**[c] [78]

[a] *Selected spectral data*: for **6**, m.p. 184–186 °C; ^{1}H NMR [$CDCl_3$–dimethylformamide (DMF), 90 MHz]: δ 1.38 (6H, 2s, *gem*-Me_2), 2.24 (3H, s, OCOMe), 2.53 (2H, s, C-3 protons), 3.38 (1H, hump, phenolic OH), 6.15 (1H, d, *J* 3 Hz, H-6) and 6.26 (1H, d, *J* 3 Hz, H-8). For **7**, m.p. 188–90 °C (decomp.); $C_{18}H_{14}O_6$, EIMS *m/z* 326 (80) [M$^+$], 283 (100), 266(27), 255 (17); ^{1}H NMR ($CDCl_3$–Me_2SO, 60 MHz): δ 2.1 (3H, s, OCOMe), 2.80 (1H, s, phenolic OH), 3.78 (3H, s, OMe), 6.10 (1H, d, *J* 3 Hz, H-6), 6.20 (1H, d, *J* 3 Hz, H-8), 7.30 and 7.80 (5H, 2m, B-ring protons). For **8**, m.p. 133–135 °C; $C_{20}H_{18}O_8$, EIMS *m/z* 386(21) [M$^+$], 344(61), 302(19), 272(5), 259(5), 179(10), 150(40), 137(61); ^{1}H NMR ($CDCl_3$, 90 MHz): δ 2.20 and 2.40 [2s, 3H each, 2 (OCOMe)], 2.70 and 3.30 (2H, 2m, C-3 protons), 3.76 (3H, s, OMe), 5.66 (1H, m, H-2), 6.02 (1H, d, *J* 3 Hz, H-6), 6.20 (1H, d, *J* 3 Hz, H-8), 6.70 (3H, m, H-2′, H-5′, H-6′) and 7.76 (1H, s, phenolic OH); UV λ_{max}/nm (MeOH): 237 and 278; NaOAc: 263 and 326. For **9**, pale-yellow viscous oil; $C_{20}H_{18}O_8$, EIMS *m/z* 386(33) [M$^+$], 356(11), 344(65), 314(7), 302(20), 272(7), 259(5), 179(9), 153(22), 150(47), 137(59); ^{1}H NMR ($CDCl_3$–DMF, 90 MHz): δ 2.28 [6H, bs, 2(OCOMe)], 2.82 and 3.04 (2H, 2m, C-3 protons), 3.82 (3H, s, OMe), 5.30 and 5.48 (1H, 2m, H-2), 6.50 (1H, bs, H-6), 6.80 (1H, bs, H-8) and 7.05–7.40 (4H, 3m, H-2′, H-5′, H-6′ and phenolic OH); UV λ_{max}/nm (MeOH): 255 and 310; NaOAc: 255 and 315. For **10**, m.p. 164–165 °C; $C_{12}H_{10}O_5$, EIMS *m/z* 234(20) [M$^+$], 192(100), 164(61), 135(6); ^{1}H NMR ($CDCl_3$–DMF, 90 MHz): δ 2.32 (3H, s, OCOMe), 2.36 (3H, s, Me), 6.15 (1H, s, H-3), 7.02 (1H, s, H-8) 7.20 (1H, s, phenolic OH) and 7.27 (1H, s, H-5): UV λ_{max}/nm (MeOH): 282sh and 330; NaOAc: 288sh and 360. [b] Ref. 12. [c] Ref. 16.

different groups of natural products, *i.e.* 5,7-diacetoxy-2,2-dimethylchromanone **1**,[9] 5,7-diacetoxy-3-methoxyflavone **2**,[10] 5,7,3′-triacetoxy-4′-methoxyflavanone **3**,[11] 6,7-diacetoxy-4-methylcoumarin **4**[12] and 4,7-diacetoxy-3-phenylcoumarin **5**[13] by lipases from porcine pancreas (PPL)§ and *Candida cylindracea* (CCL)§ in different organic solvents [diisopropyl ether (DIPE), tetrahydrofuran (THF), acetone and acetonitrile]. Among different hydrolytic enzymes, PPL and CCL have been used because they are inexpensive and their use in regio- and enantio-selective transesterifications of alcohols is well established.[14,15] By using organic solvents, we have a wide range of polarity available to achieve optimum selectivity. From preliminary screening in different organic solvents, we have found that PPL in DIPE or THF is best suited for efficient regioselective conversion.

The procedure followed for enzymatic hydrolysis was: a weighed amount of the acetylated compound (2 mmol) was dissolved in dry THF–DIPE (20–25 ml) containing n-butanol (5 equiv.), and lipase (25 mg ml^{-1} of solvent) was added. The suspension was stirred at 42–45 °C and the reaction was monitored by TLC and quenched by filtering off the enzyme after completion. The solvent was removed to dryness *in vacuo* and the product was isolated by column or thin layer chromatography. The results of the hydrolysis reactions are shown in Table 1.



The structures of compounds **6–12** obtained through the above biotransformations were confirmed from their colour reactions and spectral data (UV, ^{1}H NMR and mass spectrometry) and also by comparison of their physical data with the literature values in the case of **11** and **12**. The yields mentioned above are based on conversions measured by quantification. All the above reactions performed on different compounds under the same conditions, but without adding the enzyme did not indicate any hydrolysis.

The lipase hydrolyses predominantly the C-7 acetoxy group in hesperetin triacetate **3** giving 5,3′-diacetoxy-7-hydroxy-4′-methoxyflavanone **8** and 5,7-diacetoxy-3′-hydroxy-4′-methoxyflavanone **9** in 55 and 23% yield, respectively. The structures of **8** and **9** are based on a study of the NaOAc-induced shifts in their UV spectra. The presence of a free C-7 hydroxy group in flavanones lacking a free C-5 hydroxy group is evidenced by a NaOAc-induced bathochromic shift of band II by 40–60 nm.[17] In the case of compound **8**, a bathochromic shift of 48 nm clearly indicates it to possess a free C-7 hydroxy group, however, no NaOAc-induced shift was observed in the UV spectra of **9**, hence it was inferred to have the C-3′ hydroxy group free and the C-7 hydroxy group acetylated. Hydrolysis of **4** yielded 7-hydroxy-6-acetoxy-4-methylcoumarin **10** (65%) as the major product and 6,7-dihydroxy-4-methylcoumarin **11**[12] (15%) as the minor one. The position of the free hydroxy group in **10** at C-7 was established by observation of the NaOAc-induced shift of 30 nm in its UV spectrum. Our results indicate that in all five cases, the lipase catalyses the hydrolysis of acetoxy groups at all other positions except the one *peri* to the carbonyl group. These results are in contrast with the results obtained by chemical hydrolysis, where deacylation at the *peri* position results in the formation of a thermodynamically stable product having a free chelated hydroxy group.

In conclusion, we have shown that enzymatic hydrolysis becomes complementary to chemical deacylation as it can afford products not easily obtainable by direct chemical reactions. Thus, compounds **6**, **7**, **8**, **9** and **10** having the *peri* hydroxy or the one at the non-activated position acetylated have been prepared for the first time and of particular interest may be the formation of **8** and **9**. Hence, our study leads to selective alkylation of the C-3′ hydroxy, which by pure

§ Porcine pancreas type II lipase and *Candida cylindracea* type VII lipase were purchased from Sigma Chemical Co (USA) and used after keeping *in vacuo* over P_2O_5 for 4–5 h.

chemical means is not feasible and may prove to be of utility in providing leads to new biologically active molecules.

Received, 27th May 1992; Com. 2/02774G

References

1 J. B. Harborne, *The Flavonoids*, Chapman and Hall, London, 1988.
2 F. M. Dean, *Naturally Occurring Oxygen Ring Compounds*, Butterworths, London, 1963.
3 V. S. Parmar, R. Jain and S. Singh, *J. Chem. Res. (M)*, 1987, 2301.
4 A. C. Jain and A. K. Prasad, *Ind. J. Chem.*, 1990, **29B**, 525.
5 D. Grandjean, P. Pale and J. Chuche, *Tetrahedron Lett.*, 1991, **32**, 3043.
6 F. Bjorkling, S. E. Godtfredsen and O. Kirk, *J. Chem. Soc., Chem. Commun.*, 1989, 934.
7 D. L. Delinck and A. L. Mangolin, *Tetrahedron Lett.*, 1990, **31**, 3093.
8 M. Natoli, G. Nicolosi and M. Piattelli, *Tetrahedron Lett.*, 1990, **31**, 7371.
9 P. R. Iyer and G. D. Shah, *Ind. J. Chem.*, 1968, **6**, 227.
10 J. Kalff and R. Robinson, *J. Chem. Soc.*, 1925, 181.
11 H. R. Arthur, W. H. Hui and C. N. Ma, *J. Chem. Soc.*, 1956, 632.
12 V. S. Parmar and S. Singh, *J. Ind. Chem. Soc.*, 1987, **64**, 745.
13 V. R. Lalitha and N. V. Subba Rao, *Proc. Ind. Acad. Sci.*, 1973, **77A**, 157.
14 D. Colombo, F. Ronchetti and L. Toma, *Tetrahedron*, 1991, **47**, 103.
15 J. D. Fourneron, M. Chiche and G. Pieroni, *Tetrahedron Lett.*, 1990, **31**, 4875.
16 N. R. Krishnaswamy, T. R. Seshadri and P. J. Tahir, *Ind. J. Exp. Biol.*, 1971, **9**, 458.
17 J. B. Harborne, T. J. Mabry and H. Mabry, *The Flavonoids*, Chapman and Hall, London, 1975, p. 58.