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CATALYTIC ASYMMETRIC WEITZ-SCHEFFER EPOXIDATION PROMOTED BY BOVINE SERUM ALBUMIN. PART II.[1].

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Abstract. The epoxidation of 2 and 2,3-substituted naphthoquinones with t-BuOOH in aqueous buffer solutions, in the presence of bovine serum albumin (BSA) as chiral catalyst, affords the corresponding epoxynaphthoquinones with enantiomeric excess (e.e.) up to 79%. The influences on the enantioselectivity of the pH of the buffer solution, of the oxidizing agent, and of inorganic salts have also been examined.

Oxiranes are versatile and almost ideal synthons for the stereoselective synthesis of sophisticated compounds such as pheromones and hexose sugars.[2] They are also of fundamental importance in biological processes such as the metabolism of xenobiotics having aromatic or olefinic double bonds, the cyclisation of squalene to triterpenoids and steroids, and the mechanism of action of hormones and vitamins such as vitamin K.

Their usefulness has been improved by the Sharpless procedure[3] which simplifies the formation of chiral epoxides with known absolute configuration and high enantiomeric excess.

In the last few years we have been investigating some enantioselective reactions catalyzed by bovine serum albumin (BSA) in water as solvent, in particular the oxidation of sulphides to sulphoxides,[4] and the formation of epoxyketones from aldehydes and phenacyl halides.[5] The use of BSA as chiral template for asymmetric synthesis has been suggested to us by its analogy with the enzyme-substrate reaction. Indeed bovine serum albumin, like other carrier proteins, specifically binds and transfers a solute molecule across the lipid membrane, thus behaving like a specialized membrane-bound enzyme. More interesting, carrier proteins have different specific binding sites for the

substrate, which can be blocked by competitive inhibitors.

For these reasons BSA is a very attractive chiral catalyst, successfully used by Sugimoto in the enantioselective synthesis of sulphoxides,[6] alcohols[7] and cis diols.[8] Very recently human serum albumin (HSA) has been employed in the stereoselective photocyclisation of bilirubin.[9]

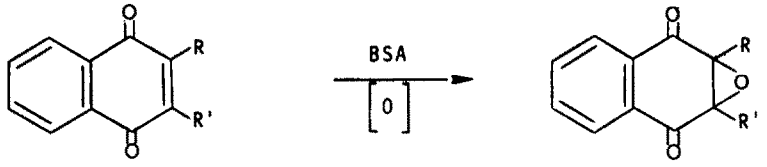
The analogy with an enzyme-substrate reaction is limited however, since the transported solute is not covalently modified by the BSA. In this context it should also be mentioned that BSA or HSA normally are not classified as proteolytic enzymes, even if they show a modest activity in the hydrolysis of p-nitrophenyl esters.[10,11]

In a preliminary communication[1], we have shown that the asymmetric Weitz-Scheffer epoxidation of 2-substituted 1,4-naphthoquinones with BSA proceeds smoothly to give the corresponding epoxides with satisfactory optical purity. The results indicated that the stereochemistry of the reaction is very sensitive to minor structural variation of the substrates, to the nature of the oxidizing species (H_2O_2 or t-BuOOH) and to steric effects.

In view of the importance of disubstituted vitamins, such as vitamins K_1 and K_2, and more generally of 2,3-disubstituted 1,4-naphthoquinones and their epoxides, we have now investigated the epoxidation of other 1,4-naphthoquinones.

Our interest was to have further information of a phenomenon of general interest, namely potentially asymmetric synthesis in the presence of proteins as chiral agents. For these reasons we have also examined the influence on the stereoselectivity of the Weitz-Scheffer epoxidation of parameters such as i) the pH of the aqueous buffer solution, ii) the nature of the oxidants other than H_2O_2 or t-BuOOH, iii) the addition of inorganic salts in order to mimic metallo-enzymes behaviour.

The reactions were carried out by stirring at room temperature a mixture of



(1-11) (12-22)

(1-12)	R = CH_3	R' = H
(2-13)	R = C_2H_5	R' = H
(3-14)	R = i-C_3H_7	R' = H
(4-15)	R = i-C_4H_9	R' = H
(5-16)	R = t-C_4H_9	R' = H
(6-17)	R = n-C_4H_9	R' = H
(7-18)	R = C_6H_5	R' = H
(8-19)	R = $CH_2C_6H_5$	R' = H
(9-20)	R = C_6H_{11}	R' = H
(10-21)	R = CH_3	R' = C_2H_5
(11-22)	R = CH_3	R' = n-C_4H_9

enones (1-11) (1 mmol) and the oxidizing agent (2 mmol) in 12.5 ml of pH 11 aqueous buffer solution containing 0.05 molar equivalents of BSA (Table 1).

The e.e. were determined by ^{1}H NMR spectroscopy with Eu(dcm)$_3$ or Eu(hfc)$_3$ as chiral shift reagents. Reaction conditions, chemical yields and e.e. are reported in Tables I-IV.

Results and Discussion

First of all we have investigated the influence of structural variations within the starting material in an aqueous buffer solution at pH 11, using H_2O_2 or t-BuOOH as an oxidant (Table 1).

The results as a whole lead to the conclusion that the highest e.e.'s are obtained with 2-isobutyl (4) and 2-cyclohexyl (9) 1,4 naphthoquinones with tert-butyl-hydroperoxide (t-BuOOH), the corresponding epoxides having 77% and 70% e.e. respectively (entries 8 and 18). In most cases examined t-BuOOH was the oxidant of choice from the stereoselective point of view.

The asymmetric induction in the catalytic epoxidation with BSA and this oxidant is generally higher than in the reaction under phase transfer conditions with H_2O_2 reported by Pluim and Wynberg[12] (entries 2,8,14,18,20,22). This tallies with the results previously reported by us in the asymmetric Darzens condensation[5] with BSA, which gave higher enantioselectivity with respect to the same reaction performed under phase transfer conditions with alkaloid onium salts as catalyst.[13]

The steric factors are particularly important: 2-tert-butyl-1,4-naphthoquinone (5) was recovered unchanged after a very long reaction time either with H_2O_2 or with t-BuOOH (entries 9,10). The same result was obtained in the attempted epoxidation of 2-methyl-3-phytyl-1,4-naphthoquinone (vitamin K_1) a (E), (Z) mixture in a 9:1 ratio.

For sterically less demanding substituents in position 2 and 3 with respect to vitamin K_1 satisfactory stereoselectivities are obtained using t-BuOOH as an oxidant, the e.e. being 54 and 48% for compounds (21) and (22), respectively, in spite of the low chemical conversions, (entries 20 and 22), which are balanced by the fact that most of the remaining reaction product is constituted by starting material.

The stereochemistry of this Weitz-Scheffer epoxidation depends on the nature of the substrates and of the oxidizing agent used. In the case of compounds (1), (3), (4) and (9) the use of H_2O_2 or t-BuOOH led to the corresponding epoxides with opposite absolute configuration (entries 1,2,5-8,17,18). In all other cases examined (entries 3,4,11-16,19,20,22) the prevailing enantiomer is independent from the oxidant. The pattern of the results does not allow any razionalisation of the sense of the asymmetric synthesis.

The enantioselectivity of the reaction is very sensitive to minor structural variation of the substrates in agreement with our previous results.[4b] Indeed in the oxidation of (4) and (6) the replacement of a i-butyl substituent by a n-butyl one, afforded the corresponding epoxides with 77% and 14% e.e.

respectively.

The vitamin K_3 2,3 epoxide (12) and by analogy the 2-alkyl substituted 1,4-naphthoquinones (13-20) with negative Cotton effect at 360 nm. have the (2R,3S) absolute configuration.[12,14]

The chemical yield in the epoxidation of 2-alkyl 1,4-naphthoquinones are generally rather low. In ancillary experiments with 2-methyl (1) and 2-ethyl (2) 1,4-naphthoquinones we have shown that for longer reaction time, with respect to those reported in Table I, the epoxidation is accompanied by competitive reactions with formation, inter alia, of the corresponding 2-alkyl-3-hydroxy-1,4-naphthoquinones, identified by comparison of their ^1H NMR and U.V. spectra with those of true samples independently prepared.

Human serum albumin (HSA) as chiral catalyst in the epoxidation of 2-cyclohexyl-1,4-naphthoquinone, in the usual condition with t-BuOOH at pH 9, affords the corresponding (-) epoxide (20) in 11% chemical yield, e.e. 70%, having the (2R,3S) absolute configuration.

Therefore, in contrast with the results obtained in the oxidation of t-butyl-(p-tolylthio)acetate with sodium metaperiodate,[4b] the same prevailing enantiomer is obtained with the two different globular proteins HSA and BSA.

The influence on the enantioselectivity of the pH of the buffer solution is worth mentioning (Table II). In most cases the enantioselectivity increases by decreasing the pH of buffer solution, as already found in the oxidation of sulphides with $NaIO_4$.[4b] This effect is more relevant for 2-methyl (1) and 2-cyclohexyl (9) 1,4-naphthoquinones (entries 23, 24 and 31, 32), while only a little variation can be observed for 2-ethyl (2) and 2-isobutyl (4) 1,4-naphthoquinones (entries 25-28). This is possibly due to the pH depending isomerization of the BSA. Indeed it has been shown[15] that at pH 9 bovine serum albumin is under a basic form "B", which is transformed into a isomerized aged "A" form for long reaction time at 30°C or by increasing the pH of the aqueous solution.

Apart from the starting substituted 1,4 naphthoquinones steric effects of the oxidizing agent can in principle play a substantial role in this asymmetric epoxidation. For these reasons we have also investigated the hitherto unreported reaction with cumyl hydroperoxide as oxidizing agent, always in the presence of BSA at pH 9.

On the basis of the results collected in Table III we may conclude that changes in the steric environment of the oxidant has a pronounced effect on the asymmetric induction, without altering the chemical yield; the oxidation with cumyl hydroperoxide of 2-methyl (1), 2-isobutyl (4) and 2-phenyl (7) 1,4-naphthoquinones afforded the corresponding epoxides with 6, 36 and 17% e.e. respectively (entries 35-37), compared to 20, 77 and 50% e.e. for the same products with t-BuOOH as oxidant (entries 2,8,14). Once more no clear trend was evident in the absolute configuration of the prevailing epoxide.

Apart from a wide variety of biological material BSA can bind cationic ligands, in particular Cu^{++} ions [16] and this could lead to an increase of the degree of asymmetric synthesis.

Therefore we investigated the epoxidation reaction with Cu^{++} ions using 2-methyl 1,4-naphthoquinone (1) as standard substrate (Table IV). The results were disappointing since the optical rotation of the obtained epoxide (12) was unchanged, when $CuSO_4$ was in 1:1 ratio with respect to the BSA.

The addition of cupric sulphate in excess with respect to the protein afforded the corresponding epoxide (12) in an almost racemic form (entry 41), whereas with cupric nitrate only starting material was recovered (entry 42).

The enantioselectivity is not substantially affected by the addition of either RuO_2 or Na_2WO_4 (entries 39, 40); in this context it should be noted that the latter metal decreases the activity of several oxidative enzymes.[17]

These results as a whole stress the limits of the analogy of BSA with enzymes, since it is well known that in metallo-enzyme metal ions can play a major role in the geometry of the protein, the positioning of the substrate and the formation of the active site, thus giving rise to a pronounced catalysis.[18]

Conclusions.

The synthetic utility of the Weitz-Scheffer epoxidation of substituted 1,4-naphthoquinones described in this paper is hampered by the levels of enantioselectivities, that compete favourably with those obtained for the same reaction performed under phase-transfer conditions, but are far from the 95% e.e. or more met with in recent advances in asymmetric synthesis. However, it has to be stressed that this reaction is catalytic, and that its interest is increased by the fact that it takes place in water as solvent and uses proteins as a source of chirality, thus resembling biomimetic processes in which optically active oxiranes are involved as intermediates.

Experimental Section

General Methods. Melting points are uncorrected. The optical rotations were determined with a Perkin-Elmer R 241 polarimeter. ^1H NMR spectra were recorded in $CDCl_3$ and the chemical shift are expressed in part per million (δ) relative to internal Me_4Si on a Varian 390 instrument. Enantiomeric excesses were determined by ^1H NMR with the aid of $Eu(hfc)_3$ or $Eu(dcm)_3$ as shift reagents by using a Varian XL 200 instrument. The CD spectra were recorded on a Jobin Yvonne Mark III dichrograph calibrated with a solution of isoandrosterone in dioxane. BSA was the fraction V Fluka commercial product.

Preparation of quinones (1-11). 2-Methyl-1,4-naphthoquinone (1) was a commercial product. Compounds (2-11), were prepared according to the literature.[12]

Epoxidation: Typical Procedure. To a magnetically stirred solution of 3.3 g of BSA (0.05 mmol) in 12.5 ml of buffer solution, the enone (1 mmol) and the oxidizing agent (eventually the inorganic salts) were added. The mixture was kept stirring for the appropriate time (see Tables) then extracted with four

portions (50 ml each) of diethyl ether. The aqueous layer was stirred overnight with 300 ml of chloroform. The slurry was filtered over a cake of celite, dried ($MgSO_4$) and evaporated. The organic phases combined were purified by chromatography on silica gel with mixture of ether and petroleum ether as eluant. The chemical and optical yields are reported in the Tables. I-IV.

Characteristics of the Epoxides.

The epoxynaphthoquinones (12-22) were all known in the optically active form, and the physical properties of our specimens were in agreement with those reported in the literature.[12]

Table I. Enantioselective Weitz-Scheffer reaction in a Buffer solution (pH 11) at 25°C.

Entry	no.	R	R'	Oxidizing agent	reaction time(days)	CD 360nm	Yield %	e.e.[a] ±2%	PTC e.e.
1	(1)	CH_3	H	H_2O_2	3	(+)	32	3	5
2	(1)	CH_3	H	t-C_4H_9OOH	3	(-)	34	20	
3	(2)	C_2H_5	H	H_2O_2	2	(+)	42	15	14
4	(2)	C_2H_5	H	t-C_4H_9OOH	2	(+)	24	5	
5	(3)	i-C_3H_7	H	H_2O_2	6	(+)	60	15	31
6	(3)	i-C_3H_7	H	t-C_4H_9OOH	6	(-)	55	21	
7	(4)	i-C_4H_9	H	H_2O_2	2	(+)	70	8	16
8	(4)	i-C_4H_9	H	t-C_4H_9OOH	2	(-)	62	77	
9	(5)	t-C_4H_9	H	H_2O_2	9		-		23
10	(5)	t-C_4H_9	H	t-C_4H_9OOH	9		-		
11	(6)	n-C_4H_9	H	H_2O_2	2	(+)	57	0	18
12	(6)	n-C_4H_9	H	t-C_4H_9OOH	2	(+)	35	14	
13	(7)	C_6H_5	H	H_2O_2	8	(-)	42	0[b]	45
14	(7)	C_6H_5	H	t-C_4H_9OOH	8	(-)	46	50[b]	
15	(8)	$CH_2C_6H_5$	H	H_2O_2	2	(-)	44	15[b]	23
16	(8)	$CH_2C_6H_5$	H	t-C_4H_9OOH	10	(-)	22	12[b]	
17	(9)	C_6H_{11}	H	H_2O_2	7	(+)	68	2	39
18	(9)	C_6H_{11}	H	t-C_4H_9OOH	7	(-)	64	70	
19	(10)	CH_3	C_2H_5	H_2O_2	14	(-)	23	11	-
20	(10)	CH_3	C_2H_5	t-C_4H_9OOH	12	(-)	22	54	
21	(11)	CH_3	n-C_4H_9	H_2O_2	14		10	0	-
22	(11)	CH_3	n-C_4H_9	t-C_4H_9OOH	14	(-)	21	48	

[a] If not otherwise indicated, the e.e. are determined in the presence of $Eu(dcm)_3$ as chiral shift reagent. [b] Determined in the presence of $Eu(hfc)_3$ as chiral shift reagent.

Table II. Effect of the pH of the buffer solution on the enantioselectivity.

Entry	no.	R	R'	oxidizing agent	pH	reaction time (days)	CD 360 nm	Yield %	e.e. ±2%
23	(1)	CH_3	H	TBHP	9	2	(-)	27	36
24	(1)	CH_3	H	TBHP	11	3	(-)	34	20
25	(2)	C_2H_5	H	TBHP	9	2	(+)	27	2
26	(2)	C_2H_5	H	TBHP	11	2	(+)	24	5
27	(4)	i-C_4H_9	H	TBHP	9	2	(-)	53	75
28	(4)	i-C_4H_9	H	TBHP	11	2	(-)	62	77
29	(6)	n-C_4H_9	H	H_2O_2	9	2	(-)	74	8
30	(6)	n-C_4H_9	H	H_2O_2	11	2	(+)	57	0
31	(9)	C_6H_{11}	H	TBHP	9	7	(-)	75	79
32	(9)	C_6H_{11}	H	TBHP	11	7	(-)	64	70
33	(9)	C_6H_{11}	H	H_2O_2	9	7	(-)	57	20
34	(9)	C_6H_{11}	H	H_2O_2	11	7	(+)	68	2

Table III. Oxidation of 2-substituted-1,4-naphthoquinones with cumyl hydroperoxide in a pH 9 buffer solution at 25°C.

Entry	No.	R	R'	Reaction time (days)	CD 360 nm	Yield %	e.e. ±2%
35	(1)	CH_3	H	2	(+)	69	6
36	(4)	i-C_4H_9	H	3	(-)	57	36
37	(7)	C_6H_5	H	7	(-)	61	17

Table IV. Enantioselective oxidation of 2-methyl-1,4-naphthoquinone (1) with t-BuOOH in presence of inorganic salts at 25°.

entry	metallic ions (mmol)		pH	reaction time (days)	Yield %	$[\alpha]_{436}$
38	$CuSO_4$	(0.05)	11	3	26	-24.1
39	Na_2WO_4	(0.05)	11	4	34	-25.5
40	RuO_2	(0.05)	11	3	37	-21.8
41	$CuSO_4$	(0.5)	11	5	47	+ 3.5
42	$Cu(NO_3)_2$	(0.5)	11	10	-	-
43	-		11	3	22	-22

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