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Intramolecular remote functionalisation of steroids by benzophenone – Increased specificity by solvent-induced hydrophobic interactions

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Abstract. Proximity of reactant sites is one of the major factors that contributes to specificity and high reaction rates observed in enzyme catalysis. Enzymes achieve this proximity between the reactant sites by having high affinity for the substrate. Structural studies on enzyme–substrate complexes provide sufficient evidence in this context and indicate that weak bonding interaction are involved in formation of such complexes. We have exploited the hydrophobic interaction between cholesterol and benzophenone to carry out photoinduced remote functionalisation of cholesterol at specific sites. Thus, using polar solvents intramolecular hydrophobic interaction between cholesterol and benzophenone permitted exclusive functionalisation of ring D in cholesterol. The current study indicates that weak interactions between the reactants can be used to bring them in proximity and photochemical reactions can provide the method for functionalising even inert sites like C–H bonds.

Keywords. Cholesterol; benzophenone; remote functionalisation; enzyme model; hydrophobic interactions.

1. Introduction

The ability of enzymes to carry out reactions at remote sites in a substrate with a high degree of specificity and that too at remarkably rapid rates, has attracted the attention of many bioorganic chemists. Several attempts have been made to simulate enzymes by making enzyme models. These models have been based on peptides as well as nonpeptide molecules and it is only of late that a peptide-based enzyme model which very nearly simulates serine proteases, has been reported (Atassi and Manshouri 1993). Similarly, other enzyme models based on different molecular receptor like cyclodextrins and crown ethers have been reported (Dugas 1989), though with a limited degree of success. However the latter studies have permitted a better understanding of enzyme action and paved the way for preparation of nonpeptide enzyme models. We report here our attempts to carry out remote functionalisation of steroids by photochemical oxidation with benzophenone and show that hydrophobic interaction between cholesterol and benzophenone can give rise to a high degree of specificity in remote functionalisation.

It is over two decades now that early reports on remote functionalisation of steroids with benzophenone appeared in literature (Baldwin *et al* 1970; Breslow and Baldwin 1970; Breslow and Kalicky 1971). The main objective of these seminal reports was

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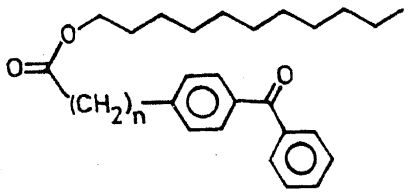
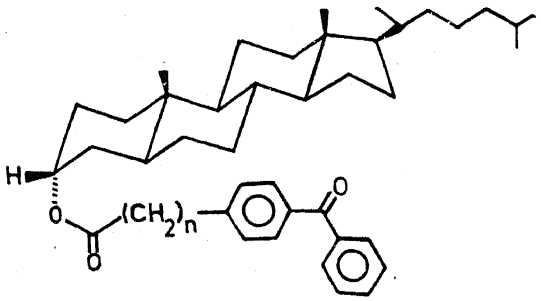
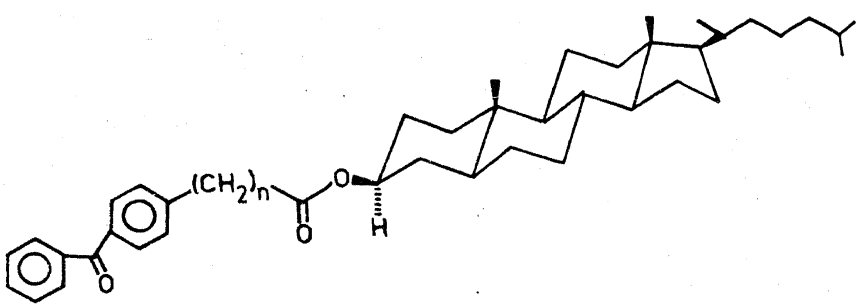
to develop alternatives to conventional stepwise functionalisation of organic compounds, involving functionalisation of neighbouring carbon atoms, to a remote functionalisation approach. In this approach, an unactivated C–H bond which is several bonds away from any functional group in the same molecule, is functionalised by choosing a conformer in which the functional group, often a precursor of a reactive intermediate generated on photolysis, and the C–H bond of interest are proximal to each other in space. This topic has been adequately reviewed by Breslow and is thus only briefly discussed here in reference to the present report, for reviews see (Breslow 1980, 1988). The early experiments (Breslow and Winnik 1969) in this direction involved intramolecular functionalisation of C–H bonds in long chain alcohols, by photolysing esters of these alcohols and 4′-alkyl-carboxy-benzophenone (I). The $n \rightarrow \pi^*$ triplet of benzophenone, formed on photoirradiation of (I), abstracts a H-radical and recombination of the resulting diradical gives rise to insertion products depending on the proximity of the C–H bond to carbonyl group of benzophenone. However the carbon atoms functionalised in the alkyl chain lacked specificity resulting in formation of several insertion products (Breslow *et al* 1978b). Similar experiments were done to carry out remote functionalisation of steroids and Breslow *et al* (1973) showed that only compounds represented by the general structure (II, 3α-isomer) give rise to remote functionalisation but not (III, 3β-isomer). Thus in conformationaly restrained (II), benzophenone carbonyl group is proximal to the α-side of cholesterol



(I)



(II)



(III)

and can therefore give rise to C–H insertion products. These experiments indicated that proximity of different groups of interest in a molecule, which is one of the major factors contributing to enhanced rate and specificity observed in enzyme catalysis (Walsh 1979), if suitably exploited can bring about reactions similar to that catalysed by enzymes without recourse to molecules based on amino acids. These early experiments thus set the tone for growth of bioorganic chemistry in reference to enzyme models.

2. Molecular design

Based on greater selectivity achieved with remote functionalisation of steroids when compared to linear alkyl chains of long chain alcohols, and possibly the commercial interest in converting cholesterol to androsterone and progesterone analogs, the interest in remote functionalisation of cholesterol has sustained with some reports appearing very recently (Breslow and Link 1992; Kaufman *et al* 1993). Most of these approaches have relied on alternative methods of functionalisation so that the covalently attached benzophenone group to $C_{3\alpha}$-position of cholesterol is replaced by other more potential functionalisation reagents capable of C–H insertion e.g. direct chlorination using phenyliodine-dichloride (Breslow *et al* 1977), radical relay chlorination (Breslow *et al* 1977; Snider *et al* 1975), oxometalloporphinates (Grieco and Stuk 1990; Stuk *et al* 1991) as cytochrome P-450 mimics and more recently manganese(III)–N,N′-*bis*(salicydeneamino)ethane with iodosylbenzene as the oxygen atom source for hydroxylation (Kaufman *et al* 1993). Some attempts to steer the benzophenone group towards Ring D of cholesterol from the β-face, have also been made using 3α,5α-bicyclo-6β-hydroxy steroids by linking benzophenone group to the $C_{6\beta}$ position rather than the $C_{3\alpha}$ position (Breslow *et al* 1984; Lee *et al* 1988). An alternative approach to improve specificity of benzophenone insertion at remote sites like ring D of cholesterol would be to exploit hydrophobic interaction between nonpolar cholesterol skeleton and benzophenone group specially when linkage between cholesterol and benzophenone is through a longer methylene chain e.g. in compounds like (II, $n = 2$ or higher). Such an approach is very likely to succeed as very effective hydrophobic interaction between cholesterol and fatty acyl chains of phospholipids have been observed in both biological and well defined artificial membranes (Yeagle 1985). However hydrophobic interaction of the type described here is unlikely to have any significance in solvents like benzene or carbon tetrachloride, solvents in which bulk of the benzophenone-based functionalisation studies have been carried out. The success of the studies carried out in the past relied primarily on using geometric constraints to achieve proximity between benzophenone and the desired C–H bond.

There has been considerable interest in improvising range of carbon atoms functionalised by benzophenone in neighbouring alkyl chains. The data is often expressed in such cases by plotting percent carbon functionalisation versus individual carbon atom of the alkyl chain (Breslow *et al* 1978b). While one observes a fairly broad range of carbon atoms functionalised in compound of type (I), in which there is very limited conformational restraint in bringing benzophenone proximal to any particular C–H bond, considerable success in selective functionalistation has been achieved by using organised media like micellar systems (Breslow *et al* 1978a;

Czarniecki and Breslow 1979). We have recently reported that by using single bilayer vesicles prepared from benzophenone-based phospholipids, very high degree of specificity in range of carbon atom functionalised in neighbouring fatty acyl chains of phospholipids can be achieved (Lala and Kumar 1993). These studies demonstrate that hydrophobic interactions can play a very important role in bringing benzophenone and the desired C–H bond close to each other. Such hydrophobic interactions, if suitably exploited, can also be used to selectively functionalise cholesterol with compounds like benzophenone. This can be achieved either by using artificial membranes prepared from cholesterol and benzophenone-based fatty acids or phospholipids, or by carrying out photolysis of compounds of general structure (II) in polar solvents as these solvents can assist in enhancing hydrophobic interaction of α-face of cholesterol and benzophenone. We describe here our results based on the latter approach.

3. Materials and methods

4-benzoylbenzene-butanoic acid was prepared by benzoylation of methylbenzene-butanoate using aluminum chloride, hydrolysed and converted to acid chloride using oxalyl chloride. The acid chloride was acylated with cholestan-3α-ol using 4-dimethyl-aminopyridine as a catalyst to get (IV). The isolated (IV) gave satisfactory spectral data. Photolysis of (IV) in different solvents was carried out in corning glass tubes in an Applied Photophysics Photoreactor using a 400 W medium pressure mercury lamp at room temperature for 2–6 h. The photolysis was followed by monitoring the disappearance of benzophenone absorption. HPLC was carried out on Shimadzu LC-4A system with a UV monitor set at 225 nm. A shimpack CLC–ODS column (6 × 150 mm) was used for analytical work with methanol as the mobile phase at a flow rate of 2 ml/min. Semi-preparative HPLC was carried out on a Altech C18 reverse phase column (10 × 250 mm). NMR spectra were recorded on a Bruker 500 MHz NMR spectrometer.

4. Results and discussion

4.1 *Molecular modeling studies*

In order to evaluate hydrophobic interaction in compounds of general structure (II), we carried out preliminary energy minimization studies using Insight II software on a SG-4D 20 work station. The initial structures were drawn using the Builder module and then minimized (molecular mechanics) with Discover module using steepest descent, conjugate gradient and quasi-Newton–Raphson algorithms. We observed that a butyric acid chain attached to 4′-position of benzophenone provided effective packing between the α-face of cholesterol and benzophenone. Thus we chose (IV) which belongs to category represented by general structure (II, $n = 3$) as the final compound to carry out our studies. The alkyl chain spacer length is just right in this compound so that both the butyroyl chain attached to benzophenone and benzophenone itself in (IV) can hydrophobically interact with α-face of cholesterol. As a result of molecular mechanics energy minimisation studies, we obtained two conformers referred to as IV–M1 and IV–M2 given in figures 1 and 2, respectively.

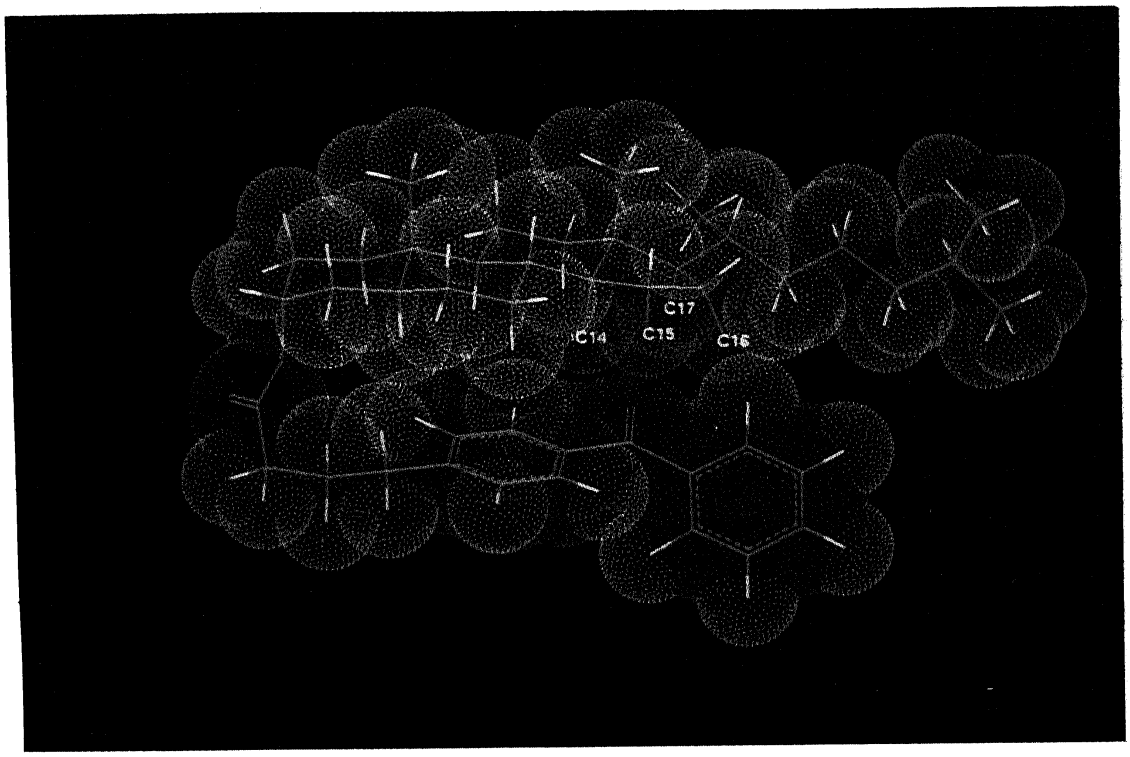


Figure 1. van der Waals surface of conformer IV–M1.

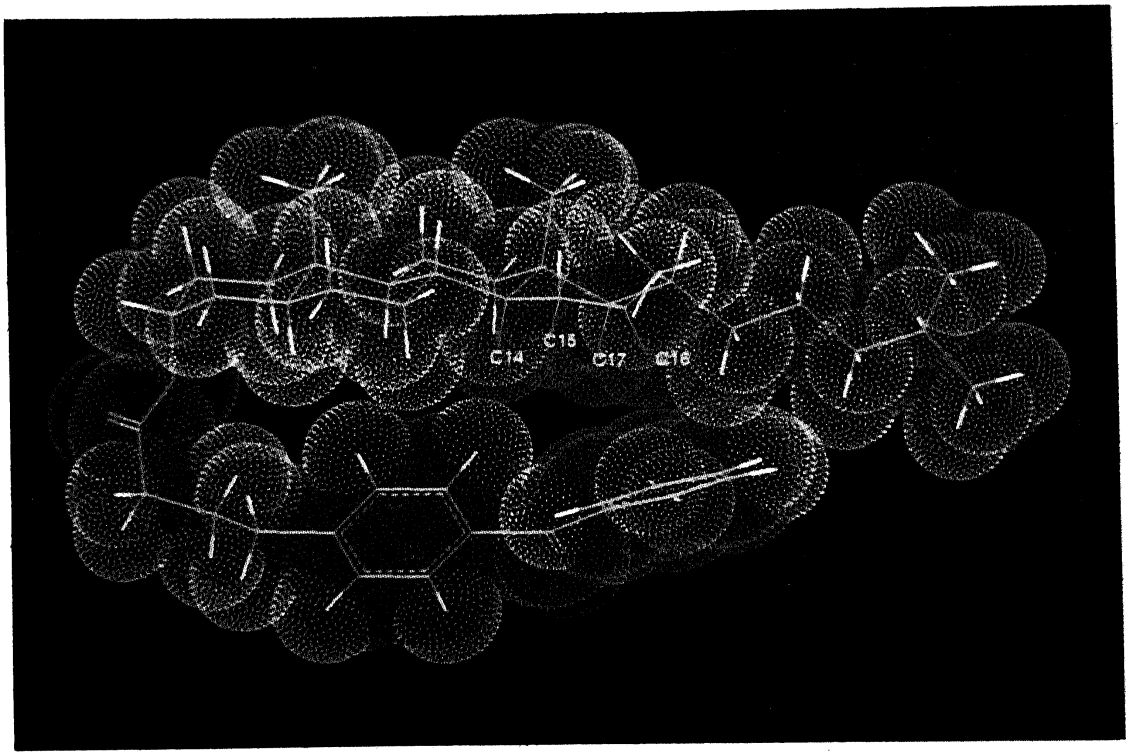


Figure 2. van der Waals surface of conformer IV–M2.

In IV–M1 one of the aryl rings in benzophenone is pointing away from cholesterol, whereas in IV–M2 it undergoes hydrophobic interaction with part of cholesterol side chain. The carbonyl group is just below the ring-D of cholesterol and figure 3 shows another view of the IV–M2, where several atoms have been deleted to highlight the proximity of benzophenone carbonyl group and the C–H bonds on the α-side of ring D. The table 1 gives distance between H-atoms of various C–H bonds in cholesterol

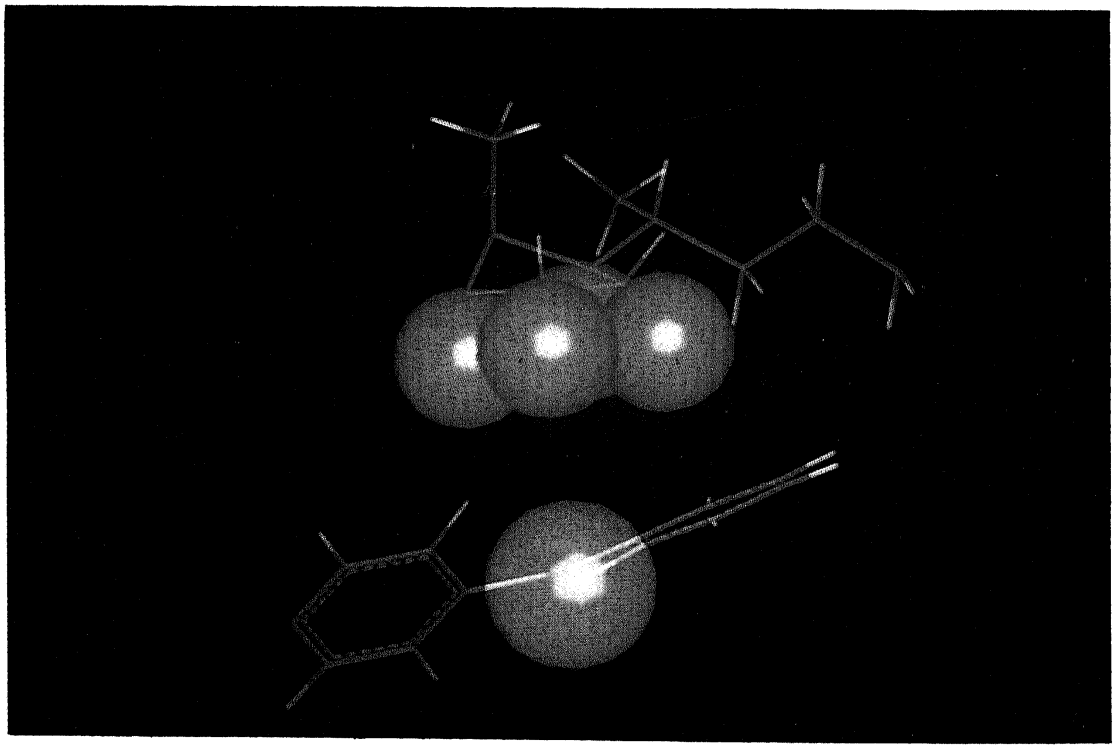


Figure 3. A truncated version of IV–M2 indicating the CPK space filling spheres for benzophenone carbonyl (red) and the four hydrogens (blue) on the α-face of ring D. Display of part of the molecule has been switched off to allow a clear view of proximity of the reactant sites.

Table 1. Distance (A) between oxygen-atom of benzophenone carbonyl group and H-atom in IV–M1 and IV–M2.

C–H bond	IV–M1	IV–M2
tert C–H		
$C_{14\alpha}$–H	3·30	4·78
$C_{17\alpha}$–H	2·48	4·19
$C_{9\alpha}$–H	4·61	5·80
$C_{5\alpha}$–H	6·53	7·26
sec C–H		
$C_{16\alpha}$–H	3·81	5·19
$C_{15\alpha}$–H	4·82	6·04
$C_{7\alpha}$–H	5·33	6·30
$C_{6\alpha}$–H	7·76	8·56

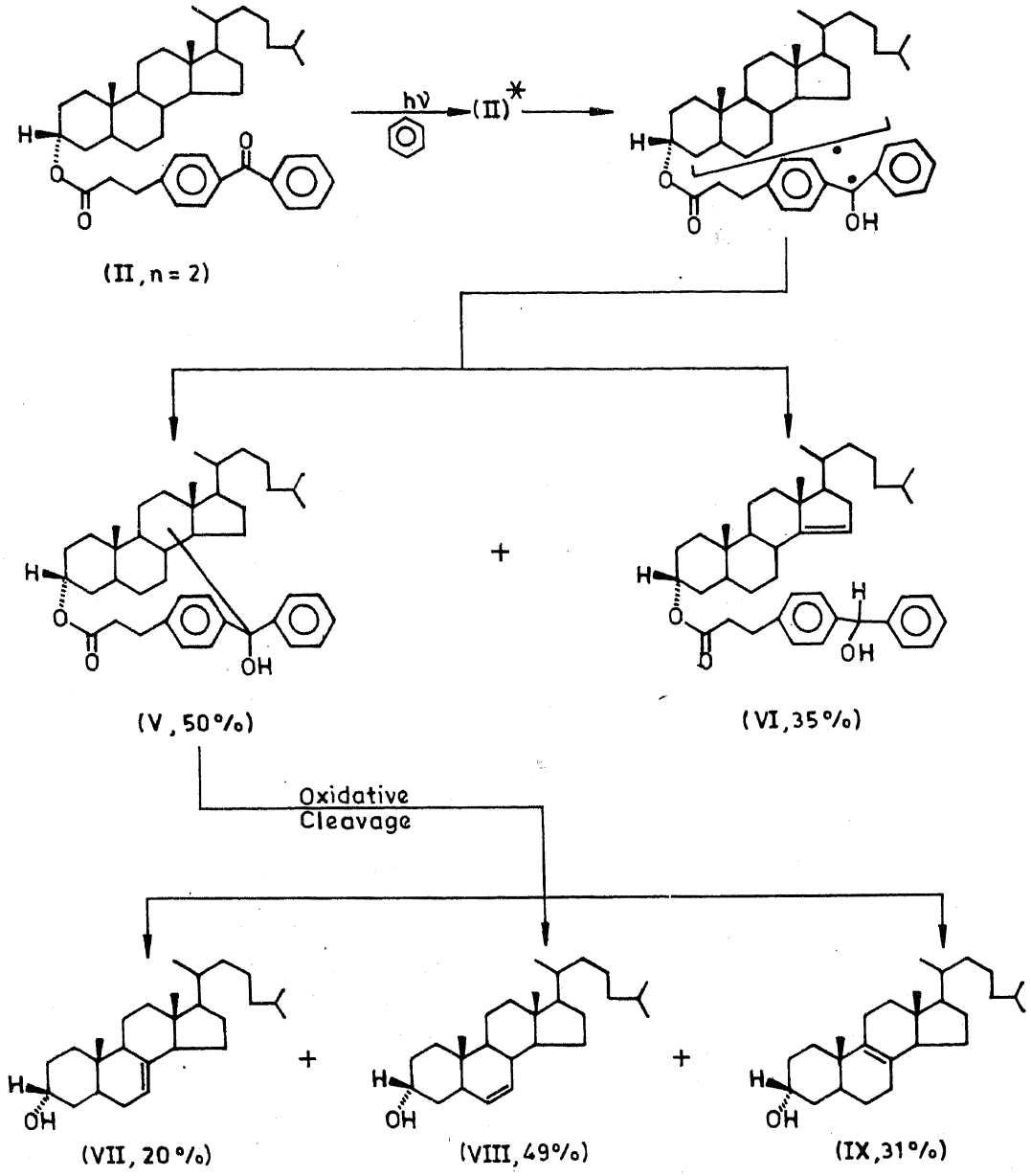
and the benzophenone carbonyl oxygen atom, indicating clearly the proximity of $C_{14\alpha}$–H and $C_{17\alpha}$–H to the benzophenone carbonyl group. The $n \rightarrow \pi^*$ triplet resulting on photolysis of benzophenone clearly shows preference for abstraction of tertiary over secondary hydrogen (Walling and Gibian 1965). Thus though the $C_{15\alpha}$–H and $C_{16\alpha}$–H are close to benzophenone carbonyl group, they are unlikely to be involved in C–H insertion.

4.2 *Analysis of remote functionalisation products*

As mentioned earlier, our main objective of pursuing the work reported here was to see the effect of hydrophobic interaction between α-face of cholesterol and benzophenone, on the specificity of products formed on photolysis of (IV). This required an analytical procedure that was both convenient and quantitative. Attempts to isolate the insertion products, though useful for the purpose of identification, can lead to experimental errors and can at best provide qualitative information. The identity of various insertion products formed upon photolysis of compounds of general structure (II) has already been established by Breslow *et al* 1973. They observed that photolysis of II ($n = 2$) in benzene led to $n \rightarrow \pi^*$ triplet of benzophenone group, which abstracts H-radical from the proximal C–H bonds giving rise to intermediate benzhydryl radical and the C-radical. This resulting diradical, then recombines to give rise to a mixture of compounds represented by lactone (V, 50%), and abstracts another proximal H-radical to give rise to Δ^{14}-ene (VI, 35%). The lactone (V) on oxidative cleavage and hydrolysis gave rise to Δ^{6}-ene (VII), Δ^{7}-ene (VIII) and $\Delta^{8(14)}$-ene (IX) (scheme 1). Thus photolysis of (II, $n = 2$) involves abstraction of several neighbouring C–H bonds on α-face of cholesterol ring skeleton. Using longer spacer connecting benzophenone and 3α-cholestanol (II, $n = 4$), the lactone yields drop and products resulting primarily from abstraction of $C_{14\alpha}$–H and $C_{17\alpha}$–H are formed i.e. Δ^{14}-ene and Δ^{16}-ene. While the identity of various insertion products was established by isolation and characterisation, Breslow *et al* (1973) used NMR spectroscopy for quantitative analysis. The olefinic protons of different steroidal olefins formed appear at distinct positions and the integration of these signals formed the basis of a quantitative analysis. However for convenience and a more quantitative analysis of the crude photolysate directly, we decided to develop a method based on HPLC analysis of the crude photolysate. Thus crude photolysate obtained on photolysis of 1 mM (IV) in benzene was analysed subjected to semi-preparative HPLC on a C18 column and individual fractions isolated. The isolated fractions were reanalysed for homogeneity and then identified by PMR spectroscopy. The HPLC and PMR data are given in table 2. Thus after being able to assign individual peaks in the HPLC chromatogram, HPLC provided a convenient and direct method for analysis of the crude photolysate.

4.3 *Specificity of remote functionalisation*

As discussed earlier, one way of exercising control on specificity of remote functionalisation in compounds of general structure (II) is to use a solvent for photolysis, which will induce hydrophobic interaction between α-face of steroid skeleton and benzophenone. The choice of benzene as a solvent for photolysis of (II), as mentioned by Breslow *et al* (1973) was dictated by the fact that benzene reacts with excited

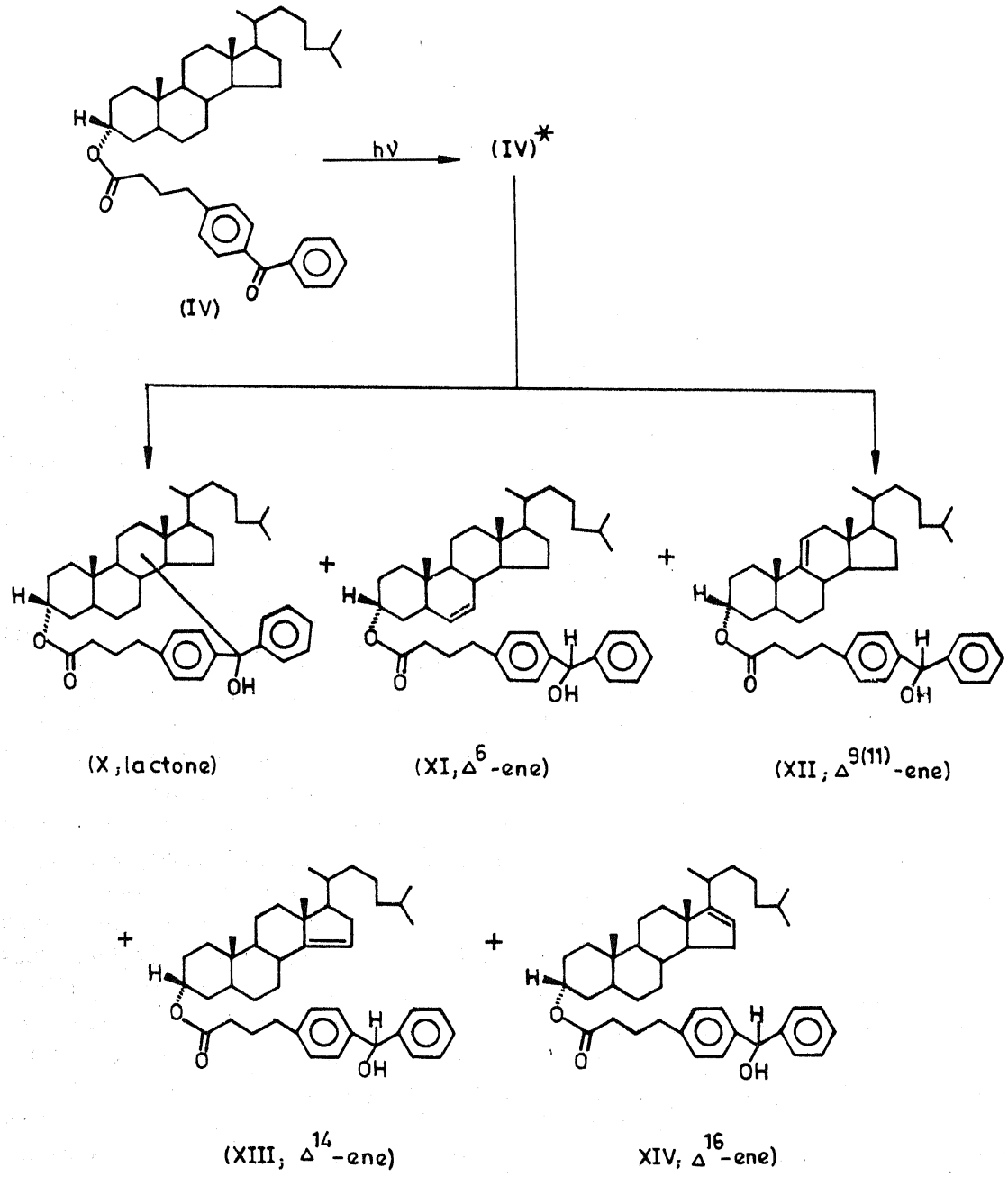


Scheme 1.

Table 2. HPLC and PMR data of fractions isolated by semi-preparative HPLC, from the crude product obtained on photolysis of (IV) in benzene.

Compound	HPLC (R_t) (min)	PMR (δ) (ppm)
(IV)	31·94	5·06 ($C_{3\beta}$–H)
(X) Lactone	11·17	4·75 ($C_{3\beta}$–H)
(XI) Δ^6-ene	16·14	5·22, 5·47 (C_6, C_7–H) (*dd*, $J = 11$ Hz)
(XII) $\Delta^{9(11)}$-ene	13·78	5·27 (C_{11}–H)
(XIII) Δ^{14}-ene	15·21	5·15 (C_{15}–H)
(XIV) Δ^{16}-ene	18·61	5·34 (C_{16}–H)

benzophenone with a quantum yield of 0·04 (Beckett and Porter 1961). Thus using benzene as a solvent at 1 mM concentration, Breslow *et al* minimised intermolecular reaction in between (II), as well as between (II) and the solvent, and obtained primarily intramolecular insertion products (scheme 1). While this result is commendable, benzene as a solvent cannot be expected to promote hydrophobic interaction between α-face of steroid skeleton and benzophenone. However polar solvents which can induce such an hydrophobic interaction are likely to themselves react with excited benzophenone $n \rightarrow \pi^*$ triplet. The choice therefore becomes limited. However we tried three water miscible solvents, acetone, acetonitrile and tetrahydrofuran, and mixtures of these solvents with water (7:3, V/V). While these solvents do show higher reactivity



Scheme 2.

Table 3. Percentage composition of intramolecular insertion products formed on photolysis of (IV) in solvents indicated, as determined by HPLC.

Solvent	Insertion products				
	Lactone (X)	Δ^{6}-ene (XI)	$\Delta^{9(11)}$-ene (XII)	Δ^{14}-ene (XIII)	Δ^{16}-ene (XIV)
Benzene	13	11	20	20	36
Acetonitrile	18	18	21	27	16
Acetonitrile: water (7:3)	18	20	5	30	27
Acetone	72	—	—	—	28
Acetone:water (7:3)	78	—	—	—	22
THF	—	—	—	49	51
THF:water (7:3)	—	—	—	—	100

towards excited benzophenone, our main objective was to evaluate the specificity of remote functionalisation by exercising control on intramolecular hydrophobic interaction in (IV). As expected, photolysis of (IV) in benzene gave number of insertion products ranging from lactone (X) to four different olefins (XI–XIV) (scheme 2 and table 3). Acetonitrile and acetonitrile:water (7:3) as solvents also gave broad insertion product range (table 3). Interestingly acetone gave exclusively the Δ^{16}-ene (XIV, 28%) as the only detectable olefin. However lactone (X) formed the major product (72%) in acetone, thus limiting the scope of direct analysis. Similar results were obtained in acetone:water (7:3) (table 3). THF gave no lactone but an almost equivalent mixture of Δ^{14}-ene (XIII) and Δ^{16}-ene (XIV). This was encouraging as it indicated resulting products almost similar to those predicted by molecular modelling studies (figures 1–3). The photolysis in THF:water (7:3) however gave exclusively the Δ^{16}-ene (XIV), as the only detectable cholesterol-based product in the crude photolysate. However this reaction is of limited practical significance as considerable amount of excited benzophenone is lost by reacting with THF. These results clearly indicate that solvents that can promote intramolecular hydrophobic interaction in (IV) can give rise to increase in specificity of remote functionalisation. Though the practical utility of results reported here is limited by reactivity of excited benzophenone towards some of the solvents used here, it does demonstrate the importance of hydrophobic interaction to achieve intramolecular proximity and reactivity. It is likely that it may not be necessary to covalently link benzophenone to cholesterol analogs e.g. (II), to conformationaly restrain the reactant sites to come close to each other. Use of well defined single bilayer membrane preparations of (i) cholesterol and benzophenone-based phospholipids (Lala and Kumar 1993) or (ii) cholesterol sulfate (which is known to form single bilayer vesicles (Brockerhoff and Ramsammy 1982) and benzophenone-based fatty acids (Lala and Kumar 1993), can provide the right molecular assembly for achieving higher degree of selectivity in remote functionalisation reactions.

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