CENTENARY LECTURE
Biomimetic Chemistry

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Biomimetic chemistry is the branch of organic chemistry which attempts to imitate natural reactions and enzymatic processes as a way to improve the power of organic chemistry. Thus, it is not so much concerned with enzyme models as aids in understanding enzymes as it is with the possibility that the imitation of natural chemistry may lead to useful new synthetic processes. While chemists have worked for many years in imitating natural pathways, as in 'biogenetic' syntheses, only recently have there been serious attempts at imitating the style of enzyme-catalysed processes in an effort to achieve some of the advantages which Nature has realized by the use of enzymes.

In attempts to imitate some of the factors involved in enzymatic catalysis, *i.e.* the formation of an enzyme–substrate complex with subsequent polyfunctional catalysis by well-placed catalytic groups (and even the incorporation of special medium effects and strain effects), the emphasis has generally been on achieving the velocity of enzyme-catalysed processes. However, this is only one of the respects in which natural enzymatic reactions have an advantage over common synthetic reactions in the laboratory. It is in many ways perhaps the less interesting aspect of natural enzymatic chemistry. For many practical purposes high speed of reaction is not as important as high selectivity, the other outstanding feature of an enzyme-catalysed process.

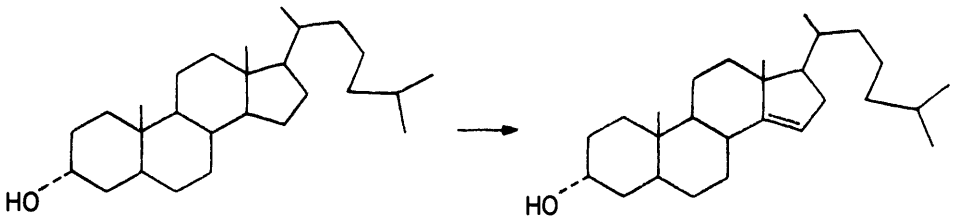
Thus, there are some enzymes which perform hydrolysis with remarkable speed, but hydrolysis is in general not a difficult problem under normal laboratory conditions for the synthetic chemist. Much more inaccessible is the sort of thing which Nature does in transforming stearic acid specifically to oleic acid, for instance, introducing a double bond into the middle of an essentially homogeneous chain of carbon atoms without benefit of any activation of these carbons by particular functional groups within the substrate. Similarly, in steroid metabolism it is quite simple for Nature to introduce double bonds or oxygen atoms in particular places, but this type of thing is not accessible to organic chemistry except by the standard, often circuitous, manipulation of functional groups.

Some years ago it occurred to us that this type of selectivity should be accessible from what was already known about the efficiency of enzyme model systems, and that it would be of great importance to try to develop a selective chemistry of functionalization at unactivated carbons. Many model enzyme systems are able to achieve accelerations by factors of 10^2 or more, and some have achieved

Selectivity

$$Me(CH_2)_{16}CO_2H \longrightarrow Me(CH_2)_7CH{=}CH(CH_2)_7CO_2H$$

stearic acid — oleic acid



cholestan-3α-ol

very large accelerations.[1] However, even a factor of 10^2 in a model system, if it is introduced because of proximity within a substrate–reagent complex, could turn an otherwise unselective functionalization reaction into a useful synthetic process. The simple acceleration of an oxidation at a particular carbon atom by 10^2 over the rate for attack at the other carbons would give synthetic conversions with much greater selectivity than organic chemists now settle for in their normal synthetic reactions. Thus, we decided to try to carry out selective functionalization using the principles of approximation which Nature incorporates into her enzymatic processes.

These principles are illustrated in a bizarre way in Figure 1, in which the particular hydrogens involved in the conversion of stearic into oleic acid are being removed by the appropriate enzyme. The factors involved are several. First of all, there is a binding site which can recognize and hold on to the end of the chain. Secondly, there is a highly reactive group which can remove unactivated hydrogens, and which is located at a well-defined distance from this binding site. It is critical that this otherwise highly reactive group be unable to attack hydrogens of the reagent itself, which in our Figure means that the monster must not be able to bite any of its own appendages, but simply the hydrogens of the bound substrate. In addition to this, an enzyme which is to functionalize a flexible substrate, such as a fatty acid, must have auxilliary binding sites so as to immobilize the chain, and these are indicated as additional hands on the creature.

Our attempts to imitate this general scheme involve the production of a rigid reagent which can attach to the end of a substrate molecule, and which cannot attack itself, but in the work done up to the present time there are at least two differences from our idealization. Our reagent is not quite so ferocious as we would like; it shows some preference for particular kinds of hydrogens over

[1] *e.g.* R. Breslow, R. Fairweather, and J. Keana, *J. Amer. Chem. Soc.*, 1967, **89**, 2135; R. Breslow and M. Schmir, *J. Amer. Chem. Soc.*, 1971, **93**, 4960.

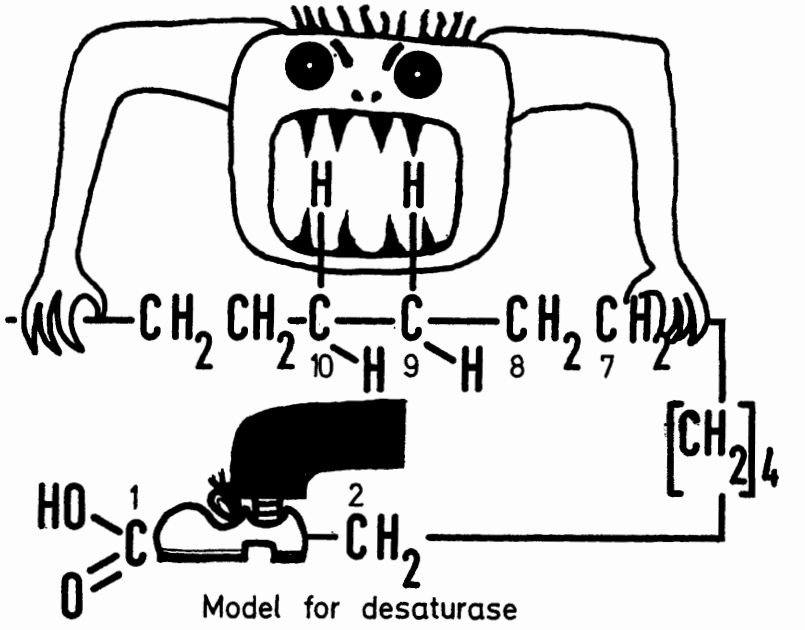


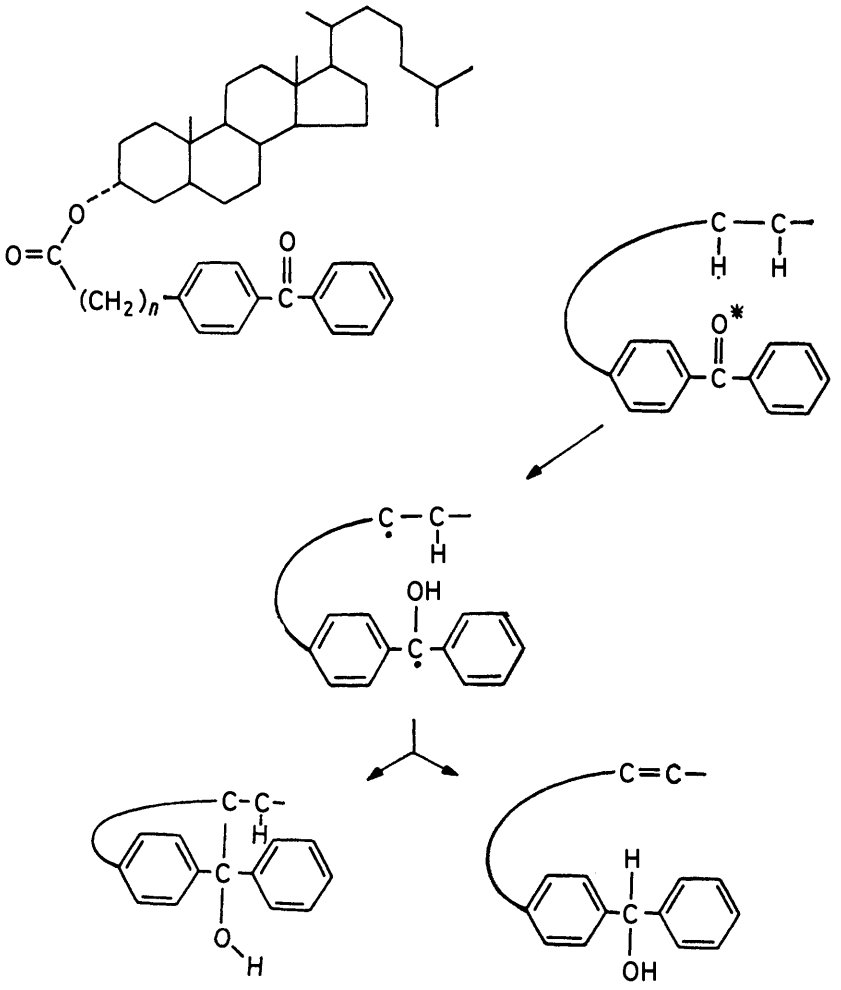
Figure 1 *A whimsical representation of the factors involved in selective enzymatic oxidation*

others, so our chemistry is not determined solely by geometry. The second difference is that we have only recently begun to work on the question of auxiliary binding sites. Thus studies which I will describe involve a 'creature' without the additional hands required to immobilize flexible substrates, or even to achieve *highly* defined orientation of reagents on to rigid substrates.

We decided to try to functionalize cholestan-3α-ol selectively. Steroids are generally available with oxygens at C-3, and the selective functionalization of steroids by biomimetic techniques would be of considerable practical interest. Models convinced us that it was desirable to try to functionalize steroids on the α or bottom side, which is considerably less hindered in comparison to the top on which the angular methyl groups form a barrier to close approach. For the rigid reagent we initially selected various benzophenone carboxylic acids. The first approach involves forming an actual ester in the attachment of the reagent to the substrate, but this covalent interaction is simply a convenience and is not necessarily required, as we will indicate later. The benzophenone carboxylic acid esters have the great advantage that benzophenone can be photo-excited to its triplet state, in which it is able to attack unactivated methylene and methine groups, and it cannot attack itself.

The chemistry expected is illustrated in Scheme 1. The oxygen atom of the

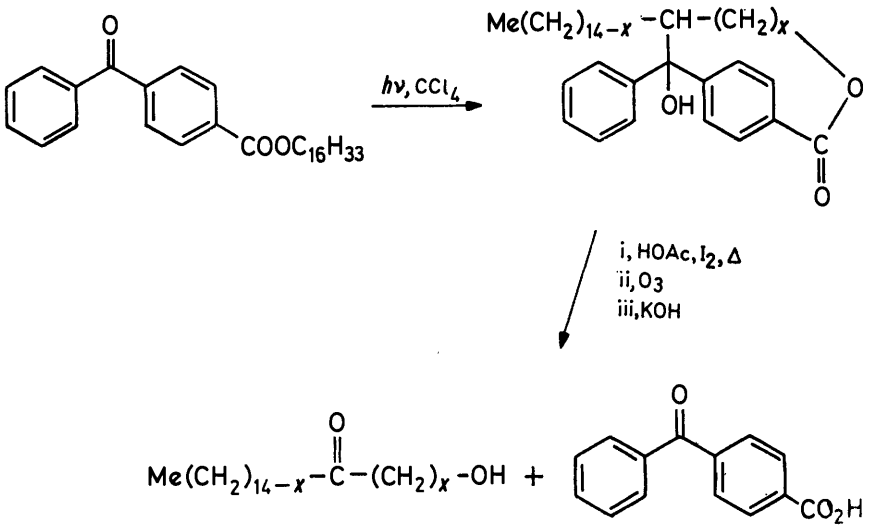
benzophenone triplet should attack a particular hydrogen or set of hydrogens in the substrate to form a diradical, which will still be held together by the link between reagent and substrate. This diradical can thus either collapse to form a new carbon–carbon bond, or undergo hydrogen transfer to form a double bond in the substrate and a reduced carbonyl group in the benzophenone. In principle, hydrogen atom transfer could also occur to regenerate starting materials, but this will only slow the reaction. We have no evidence for major amounts of such a process.



Scheme 1

After some preliminary examination of steroid cases, we decided to explore the scheme first with flexible substrates. Thus, a series of straight-chain esters of benzophenonecarboxylic acid were prepared and photolysed.[2] The products from these reactions result from attack by benzophenone oxygen on a substrate hydrogen and subsequent collapse of the diradical to form a new carbon–carbon bond. No appreciable amount of olefin has ever been detected in our studies of these flexible substrates, but some of the carbon–carbon coupling occurs into the aromatic ring.

It was easily possible to determine the position which had been attacked, since the resulting alcohol can be dehydrated under mild conditions and the new double bond to the functionalized carbon can be cleaved with ozone (Scheme 2).

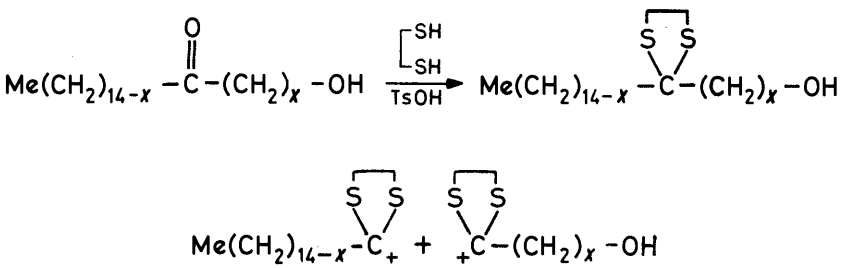


Scheme 2

After hydrolysis the benzophenone carboxylic acid is regenerated (making the overall process formally a catalysis) and the product keto-alcohol mixture could be examined to determine where functionalization had occurred. Although various methods[2] were examined to determine this, the most reliable seems to be a recent procedure[3] in which we convert the resulting carbonyl group into a

[2] R. Breslow and M. A. Winnik, *J. Amer. Chem. Soc.*, 1969, **91**, 3083; M. A. Winnik, Ph.D. thesis, Columbia University, 1969.
[3] R. Breslow and P. C. Scholl, *J. Amer. Chem. Soc.*, 1971, **93**, 2331.

thioketal with ethylenedithiol and then examine the mass spectrum of the resulting product mixture (Scheme 3). At low ionizing voltages mass spectral fragmentation occurs very largely at the carbon carrying the sulphurs, and from the pattern of mass spectral peaks it is simple to determine the distribution of oxidation sites.



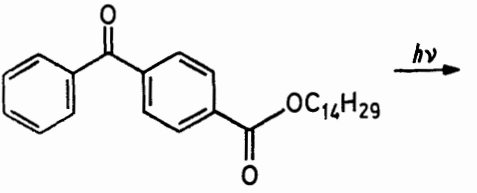
Scheme 3

The results for a relatively short chain of 12 carbons are as shown in Table 1, *i.e.* a rather selective functionalization of the 11th carbon, with some attack at carbon 10 and only negligible amount of attack much closer to the point of attachment. Models indicate that attack closer than carbon 8 should not be possible in this process even if the chain is fully extended, and this has been confirmed in all of our studies. Thus, in this relatively short chain we have achieved quite selective functionalization without additional binding sites to immobilize the chain.

Table 1

Carbon	%
8	1
9	6
10	28
11	65
12	0

However, if the chain is made longer the flexibility of the system introduces more randomness in the positions of functionalization. Thus, with a fourteen-carbon chain (see Figure 2), there is again no functionalization closer than carbon 8, but now we get more than 10% attack on carbons 10, 11, 12, and 13, with the maximum at carbon 12 of approximately 50%. If the chain is made



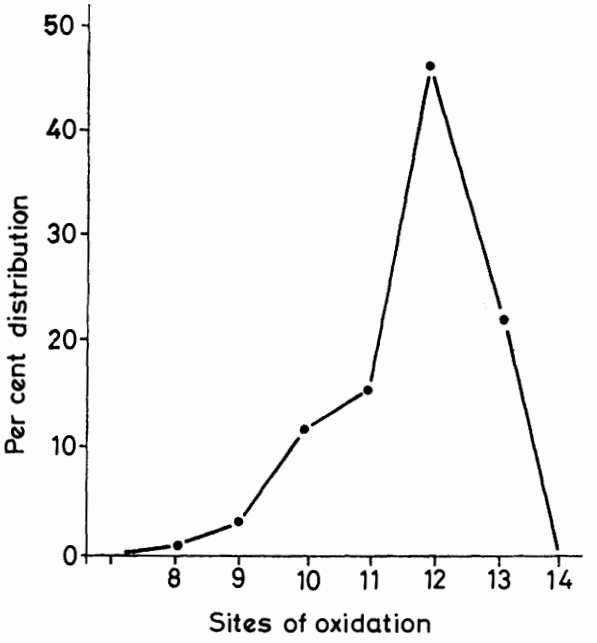


Figure 2 *Photo-oxidation of the* C_{14} *ester*

still longer, then a similar pattern persists, but with a greater distribution. Thus, the eighteen-carbon chain produces a broad distribution covering carbons 10 to 17 (see Figure 3); again there is no attack closer than carbon 8, and no attack on the terminal methyl group. The absence of attack on the methyl is apparently the result of the chemical selectivity of the benzophenone triplet, since the methyl hydrogens are less reactive than are methylene hydrogens. In this respect our reagent is letting chemical selectivity come into the reaction, as well as the geometric selectivity we are building in.

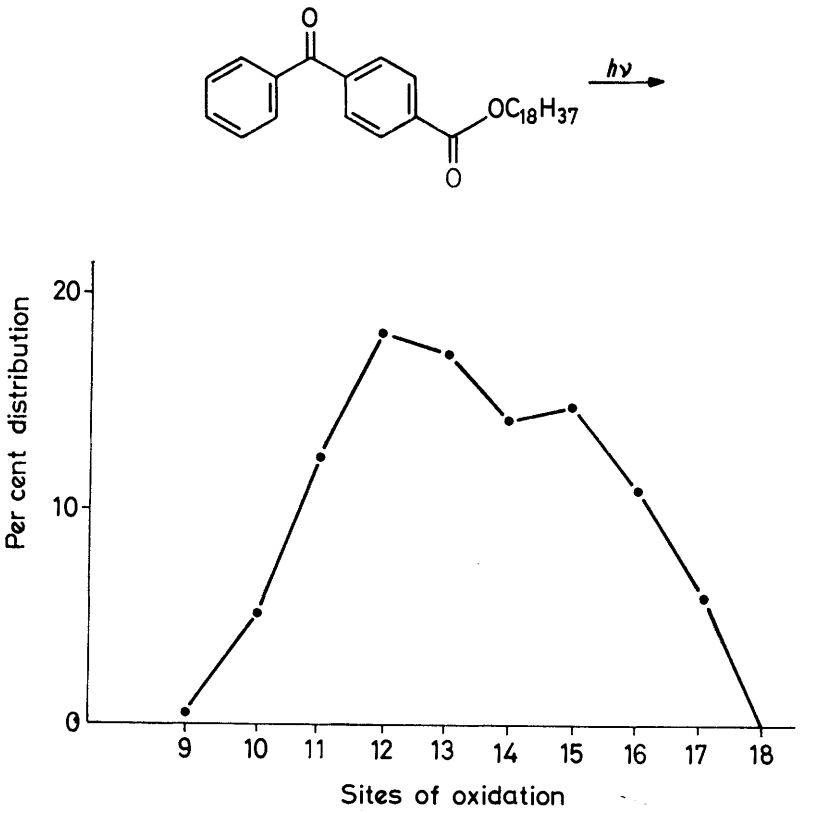


Figure 3 *Photo-oxidation of the* C_{18} *ester*

Although in the eighteen-carbon case this kind of distribution can hardly be considered synthetically interesting, the technique is an attractive way to determine conformations of flexible chains under various conditions. Thus, we have found in some other solvents that the eighteen-carbon chain becomes highly coiled and we achieve rather good selective attack on carbon 17. This type of study is of considerable interest in understanding the interactions of flexible chains with their environment, but it does not move us further toward our goal of highly selective preselected reaction at particular carbons. Only subsidiary binding sites are likely to make this possible with the flexible systems.

With steroids there is much less of a problem. As Figure 4 shows, there are only a limited number of accessible axial hydrogens on the bottom of a steroid, and one might expect to be able to attack particular ones of these selectively if

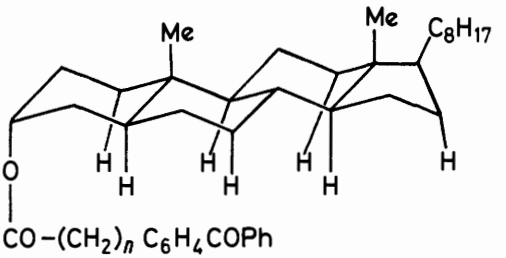


Figure 4 *A conformational representation of the steroid esters of our benzophenone acids*

the reagent is carefully designed. Models show that it is not possible to use a simple benzophenone-4-carboxylic acid ester of cholestan-3α-ol, since the rigid reagent and rigid substrate form a V in which the carbonyl oxygen cannot reach the steroid hydrogens. This is confirmed both by the lack of any photochemistry of the type to be described shortly, and also by the finding that this molecule has a long phosphorescence lifetime,[4] indicating that there is no process by which the benzophenone triplet is appreciably perturbed by the steroid.

The phosphorescence lifetime τ of a benzophenone triplet depends on several factors, including quenching (whose rate will be $k_q[Q]$):

$$^3Ph_2CO \xrightarrow{k_d} Ph_2CO \qquad \text{radiationless decay}$$

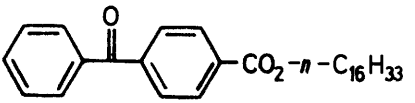
$$^3Ph_2CO \xrightarrow{k_p} Ph_2CO + h\nu \qquad \text{phosphorescence}$$

$$^3Ph_2CO + RH \xrightarrow{k_r} Ph_2\text{-}\dot{C}OH + R\cdot \qquad \text{hydrogen abstraction}$$

$$\tau_{Ph_2CO} = \frac{1}{k_d + k_p + k_q[Q]}$$

Hydrogen abstraction from a substrate, reversible or not, is one form of quenching. Figure 5 shows phosphorescence data for n-hexadecyl *p*-benzoylbenzoate in comparison to data for an intermolecular control, an equal mixture of methyl *p*-benzoylbenzoate and hexadecyl acetate. The data are listed in Table 2. The long intermolecular τ's are shortened in intramolecular cases in which hydrogen transfer can occur. Figure 6 illustrates an additional control.

[4] T. Flechtner, unpublished work.



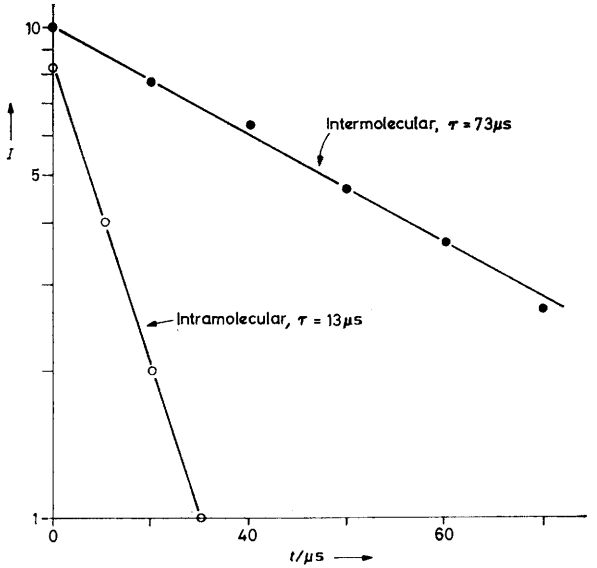
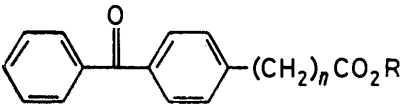


Figure 5 *The determination of phosphorescence lifetime for a benzophenone derivative which can undergo intramolecular hydrogen transfer compared with an intermolecular control in which the benzophenone and the alkyl chain are not attached*

If the linkage between the benzophenone system and the steroid system is made somewhat longer, the V can be transformed to a U in which the two parallel sides can now be stacked on each other, and hydrogen transfer is possible in the triplet. The first case examined[5] was the benzophenone-4-propionic ester of cholestan-3α-ol (1), which was irradiated in various solvents. Models indicated that the carbonyl oxygen in this compound can swing in an arc which passes under carbons 7, 14, and 12, so it was expected that these three positions might be functionalized by the reagent.

[5] R. Breslow and S. W. Baldwin, *J. Amer. Chem. Soc.*, 1970, **92**, 732, and subsequent unpublished work.

Table 2 *Triplet lifetimes*



$(10^{-3}$ mol l^{-1} in CF_2ClCCl_2F, 25°C)

R	n	$\tau_{inter}/\mu s$	$\tau_{intra}/\mu s$
Cholestan-3α-ol	0	73	68
Cholestan-3α-ol	2	65	10
Cholestan-3β-ol	4	20	15
Hexadecan-1-ol	0	73	13

Attack at position 14 is confirmed by two observations. The first is that a certain amount of unsaturated steroid is formed directly. The carbonyl oxygen of the benzophenone triplet removes the hydrogen at carbon 14, and the carbon atom of the benzophenone then removes a neighbouring hydrogen. There are only two neighbours with hydrogens, C-8 and C-15, and the hydrogen at C-8 is on the top, or β, side of the steroid. There is no choice for the reagent, which is attached on the α side, but to remove the α hydrogen at C-15, producing the cholest-14-enol ester directly as the only unsaturated steroid product. In addition to this, the same diradical undergoes some collapse to form a new carbon–carbon bond at C-14. This is indicated by the observation that this alcohol, which of course cannot be dehydrated and oxidized as in our previous degradation, can be cleaved with lead tetra-acetate to produce cholest-8(14)-enol (Scheme 4). It is striking that in this case the attack involves oxidation to generate the same C-14 radical by lead tetra-acetate cleavage, but then the hydrogen atom which is removed by an external random reagent comes preferentially from C-8, because there is no stereochemistry of an internal complex with reagent to dictate the direction of the hydrogen atom removal.

In addition to these products from attack at C-14, there is steroid which has been attacked at methylene groups with subsequent coupling. In this material the alcohol fraction can be dehydrated. The sequence used involves cleavage with ruthenium tetraoxide and sodium periodate rather than ozone, since ozone forms the epoxide. The result of this process (Scheme 5) was the formation of two ketones, of which the first recognized and identified[5] was 12-ketocholestan-3α-ol and the other is 7-ketocholestan-3α-ol.

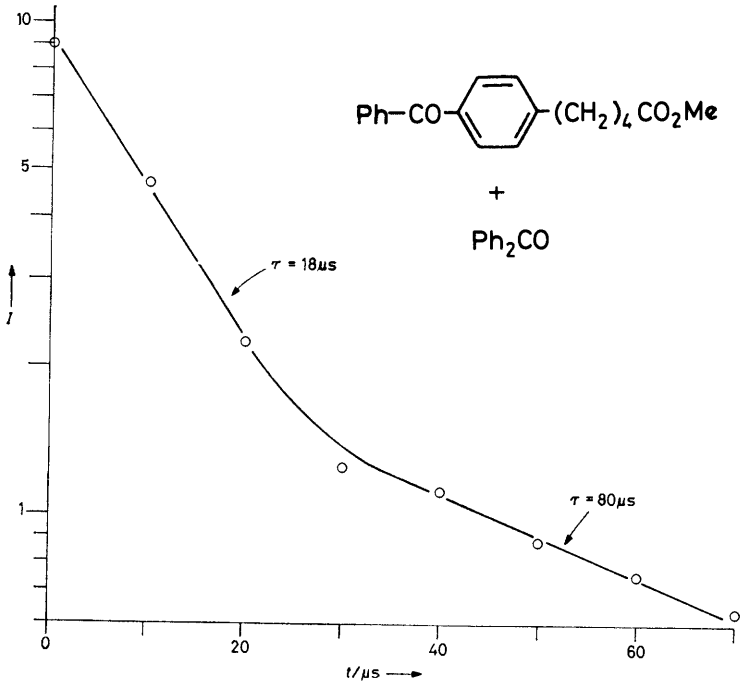
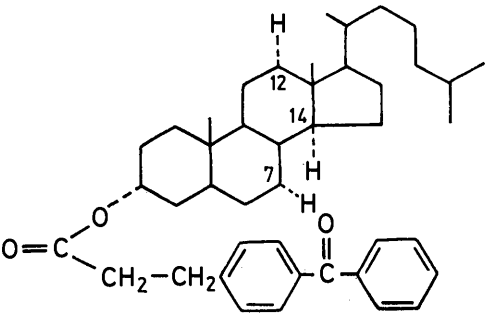
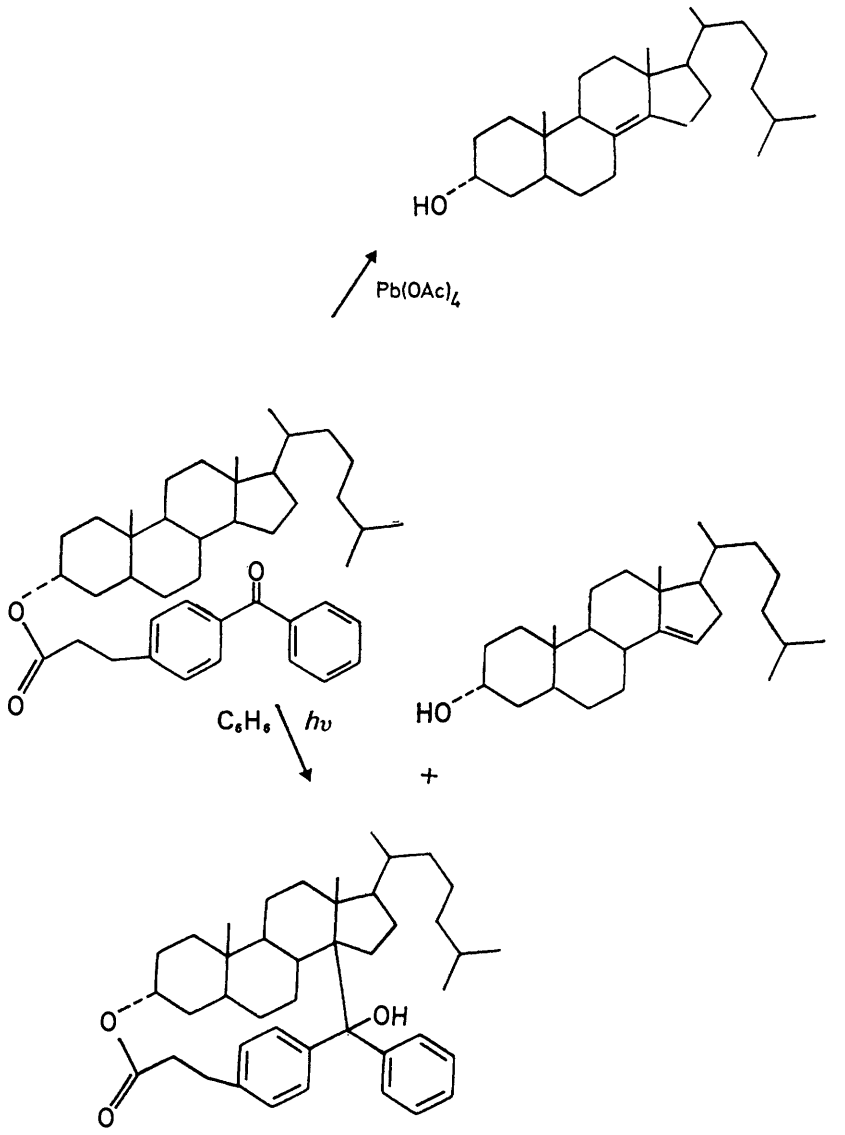


Figure 6 *The simultaneous determination of phosphorescence lifetime for benzophenone and for a benzophenone derivative which undergoes internal quenching (without overall photoreaction)*

Thus, these results and other studies we have done on this reaction indicate that the prediction of the model is precisely correct: the three hydrogens attacked



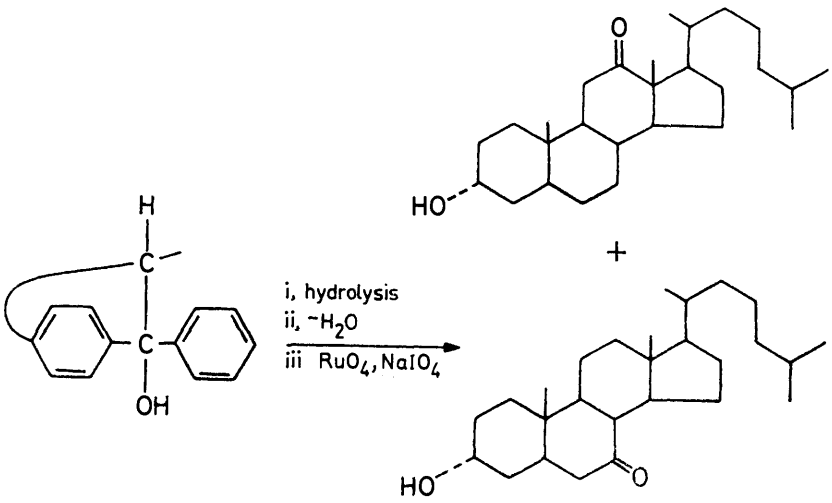
(1)



Scheme 4

are those at C-7, C-14, and C-12. This situation can be changed by altering the geometry of the reagent.

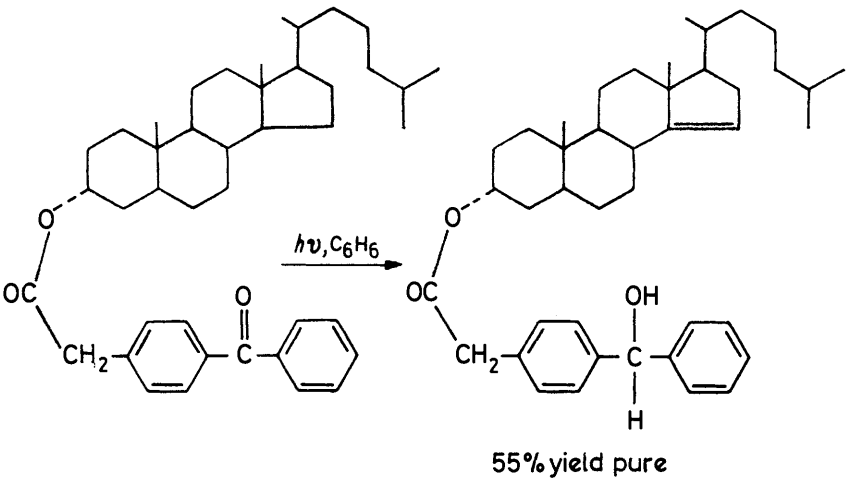
Perhaps the most striking change occurs on going to the benzophenoneacetic acid ester of cholestan-3α-ol.[4] On irradiation, this compound undergoes selective attack at C-14, and the product of reaction of the diradical is exclusively the olefin resulting from hydrogen atom transfer from C-15. In fact, the only detectable steroid product, formed in 55% yield, is the cholest-14-enol (Scheme 6). The remainder of the material has undergone reduction of the benzophenone by solvent, but the steroid is recovered unchanged. Thus, in this case, there has been increased selectivity for initial attack at C-14. Perhaps most striking, the shorter link between the two rigid systems means that collapse to form a new



Scheme 5

carbon–carbon bond is apparently too strained, so hydrogen atom transfer is the exclusive process which the diradical undergoes. This procedure represents an attractive synthesis of cholest-14-enol, and it can be run to produce several grams of this material at a time.

Extension to a longer link between the two rigid systems might at first have been thought to lead to some randomness. However, various of our studies have led us to the picture that with short segments of methylene chains the geometry of these systems has a bend at the ester group and then a more or less extended segment of chain. This results in the benzophenone system again packing on the bottom of the steroid but being moved further into ring D. Thus, in the system



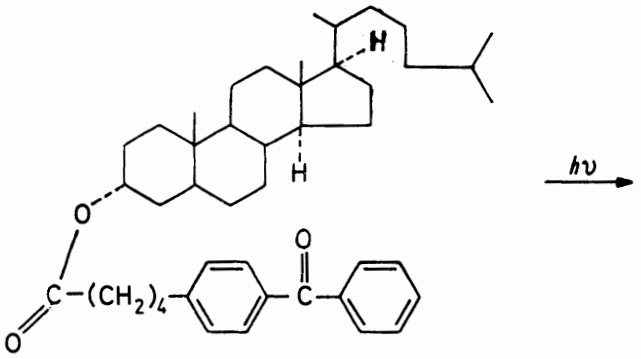
Scheme 6

with four methylene groups,[6] the benzophenonepentanoic acid ester of cholestan-3α-ol (2), the triplet oxygen attacks hydrogens at C-14 and C-17. Again the subsequent reaction is chiefly hydrogen atom transfer (Table 3). For the reasons described previously, the C-14 radical leads to the Δ^{14} olefin. The C-17 radical, again selectively, transfers the hydrogen at C-16 to produce cholest-16-enol after hydrolysis of the reaction products. Only a very small distance lies between the 14 and 17 hydrogens, which are essentially 1,3-diaxial, and subtle changes in the conformation of the material in different solvents alter the selectivity for abstraction between the two positions. Thus, even without additional binding sites, manipulation of solvents and conditions can have some useful effect in producing selectivity.

Studies have been done of other steroid derivatives.[7] Thus, if we attach a benzophenonehexanoic acid at C-17 of ring D, even though the attachment is on the β-side, the reagent can curl under the steroid and pack on the bottom of the steroid (Scheme 7). Models indicated that with this number of methylenes the carbonyl oxygen can get down into the centre of ring C. Indeed, this benzophenone triplet attacks hydrogens at C-9 and C-14, two protons which are 1,3-diaxial on ring C. The product from removing the hydrogen at C-14 is again directly the cholest-14-enol, but in the case of hydrogen abstraction at C-9 no direct olefin is produced. The neighbouring hydrogen at C-8 is, of course, β, while the hydrogens at C-11 are quite crowded and difficult to reach. Thus, this diradical collapses to form the new carbon–carbon bond to C-9. Lead tetra-acetate cleavage and hydrolysis produces the cholest-9(11)-enol as the

[6] *Cf.* also J. E. Baldwin, A. K. Bhatnager, and R. W. Harper, *Chem. Comm.*, 1970, 659.
[7] R. Breslow and P. Kalicky, *J. Amer. Chem. Soc.*, 1971, **93**, 3540.

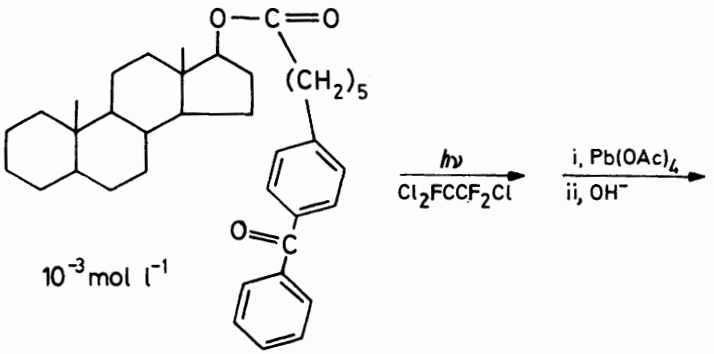
Table 3



(2)

Solvent	*Olefin Yield*	$\Delta^{16}:\Delta^{14}$
Benzene	65%	1.3
HOAc	70%	2.1
Acetone	66%	4.1
(−40 — +50°C)		

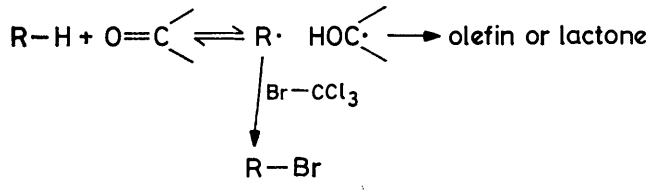
only product of oxidative cleavage. In this way it is possible to introduce the 9—11 double bond into a steroid and accordingly to gain entry into the important 11-oxygenated corticosteroid series.



55% starting steroid + 25% olefin (80% $\Delta^{9(11)}$, 20% Δ^{14})

Scheme 7

The kind of chemistry just described represents a rational attempt to use orientation to achieve this important introduction of a 9—11 double bond into steroids. Curiously, at the same time we discovered[8] that some other techniques can be used to achieve this process. In the course of our studies of remote oxidation with benzophenone derivatives we had observed that chlorosteroids were produced occasionally when carbon tetrachloride was the solvent. Reasoning that this might involve the capture of the intermediate diradical by solvent, we decided to try to intercept this radical with a more effective radical trap. Thus, we introduced $BrCCl_3$ into our remote oxidations, and found that indeed the products were largely diverted to bromosteroids and no carbon–carbon insertion reactions had occurred (Scheme 8). However, the principal position of



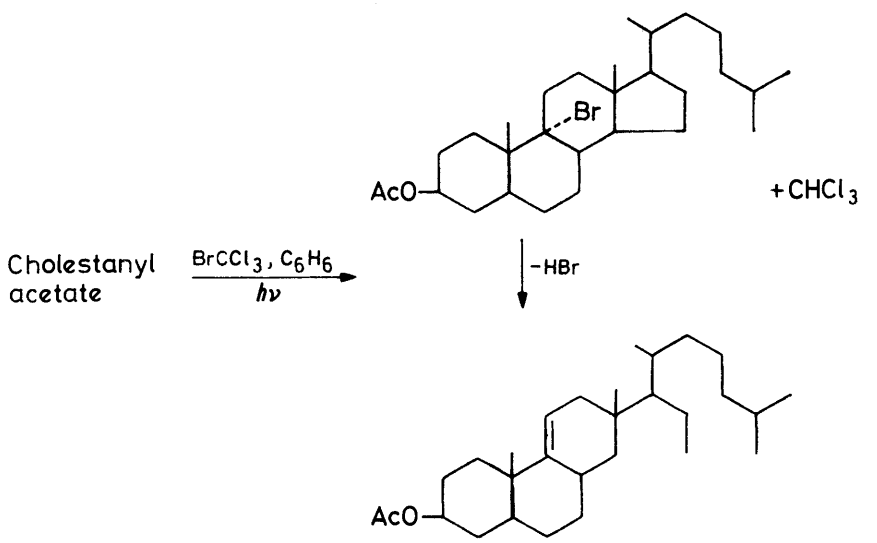
Scheme 8

bromination was in fact C-9, a position which was not otherwise attacked by such benzophenone derivatives as the benzophenoneacetic ester of cholestan-3α-ol.

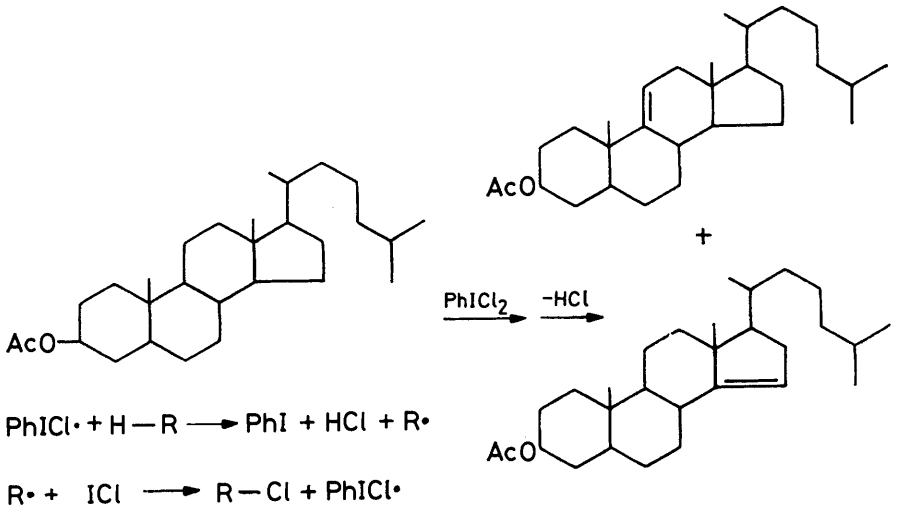
Controls quickly established that this was the result of a direct free-radical halogenation of the steroid, sensitized by the benzophenone, and that other sensitizers could be substituted. Thus, the direct short-wavelength irradiation of a benzene solution of $BrCCl_3$ with cholestanyl acetate also afforded the 9-bromocholestanyl derivative as the major product (Scheme 9). Although this process looks useful, it can only be carried to low conversion because the product is attacked by the reagent. More attractively, $PhICl_2$ performs selective chlorination of such compounds as cholestenyl acetate (Scheme 10). In this case, a 75% yield of chlorosteroid can be produced as a one-to-one mixture of the 9-chloro- and 14-chloro-steroids. With other derivatives of $PhICl_2$ it is possible to improve the selectivity for functionalization at C-9.

These free-radical processes thus seem to be procedures which can be developed into attractive chemical ways to introduce the functionality in ring c needed for the production of corticosteroids. However, they cannot be general substitutes for the oriented biomimetic oxidations we have been describing, since they depend on a hitherto undetected selective reactivity of the tertiary axial hydrogen at C-9. It is possible that even in these cases the process is directed to some extent by orientation factors. The flat reagents, CCl_3· and PhICl·, may

[8] R. Breslow, J. Dale, P. Kalicky, S. Liu, and W. Washburn, *J. Amer. Chem. Soc.*, 1972, **94**, 3276.



Scheme 9



Scheme 10

selectively pack on the bottom of the steroid so as to minimize the total volume excluded from the solvent; thus they may be directed by packing forces to the positions attacked. Further work will be required to explore this possibility.

Naturally, there is considerable interest in determining whether the principles of orientation and directed attack which we have utilized in benzophenone chemistry can be generally applied to functionalization reactions. One of the best-known functionalization processes is the Barton reaction.[9] In this reaction, a nitrite ester, on photolysis, produces an oxygen radical which attacks nearby hydrogen atoms to functionalize unactivated carbons over small distances. The product after hydrogen atom transfer captures NO to produce the corresponding nitroso-compound. There are many other related processes, such as the chemistry described by Heusler.[10] Our process of remote oxidation with benzophenone involved taking the well-known ability of triplet carbonyl groups to attack nearby hydrogens, and extending this to very large distances by the use of rigid separation by reagent and substrate. Thus one would think that this extension could also be applied to reactions such as the Barton process.

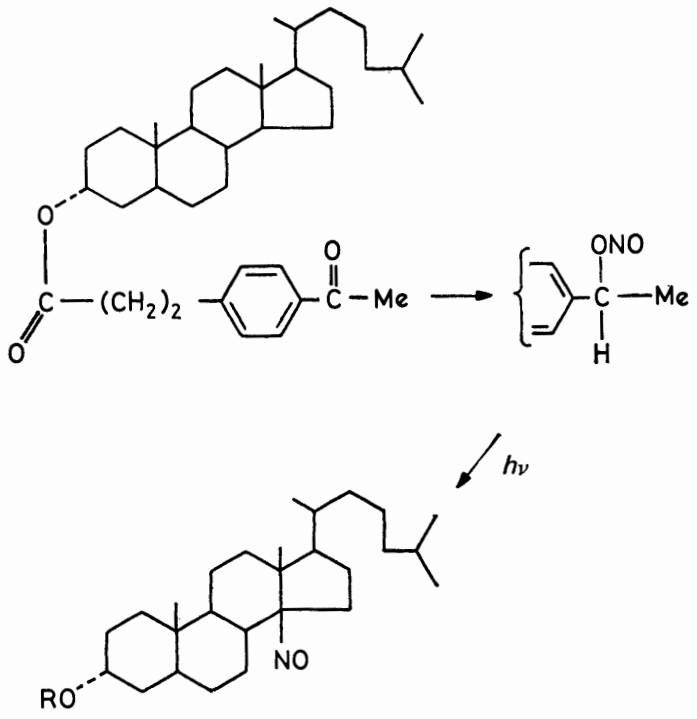
The only evidence we have on this so far is the case illustrated in Scheme 11. On irradiation, the nitrite ester gives a modest yield of nitroso-steroid, together with considerable recovered unfunctionalized steroid. However, the functionalization appears to be selective at C-14; the resulting nitroso-steroid can be reduced to the 14-β-aminocholestan-3α-ol.

The other direction in which it would be important to extend our observations is to the use of complexing, rather than covalent attachment, between reagent and substrate. Again, many possible kinds of interaction can be visualized. We have worked this out in only one case[3] and established that it can be achieved.

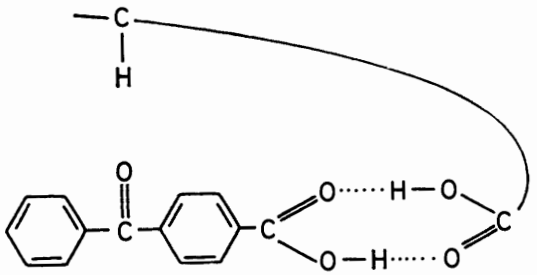
The proposition is a fairly general one. One way to describe the type of chemistry we are doing is to say that a reagent which could ordinarily come up to a substrate in a fairly random manner, and attack a variety of carbons, has a preference for attack on a particular carbon in our systems because in the transition state for attack there is an extra interaction of a binding sort which stabilizes that particular transition state. The extra interaction we have used up until now is a covalent ester linkage, in which case the description just given is a bit far fetched. However, in general, if a binding interaction of a few kilocalories could lower the energy of one of the transition states, one might expect to get selective reaction without necessarily requiring the very large number of kilocalories of a covalent linkage.

The case we have examined uses the double hydrogen bond of a carboxylic acid dimer (Scheme 12). If a substrate carboxylic acid and a reagent carboxylic acid are together in non-polar solution three species will in general be present. Each of the carboxylic acids can form its own dimer, and in addition there will be the mixed complex between the two. Using benzophenone photochemistry, the only possible reaction will involve the mixed complex, and as it is removed

[9] R. H. Hesse, *Adv. Free Radical Chem.*, 1969, 83.
[10] K. Heusler and J. Kalvoda, *Angew. Chem. Internat. Edn.*, 1964, **3**, 525.



Scheme 11

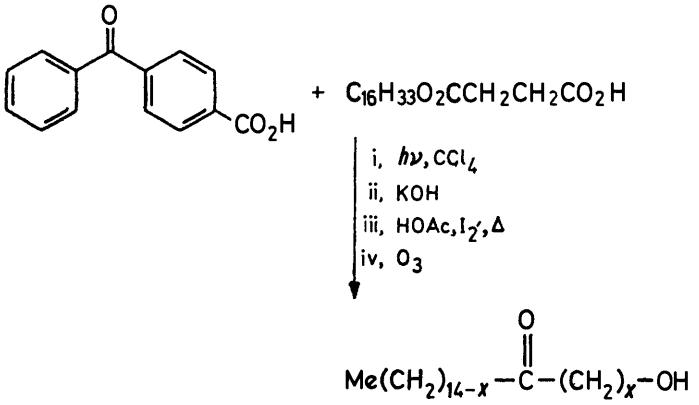


$$RCO_2H + R^1CO_2H \rightleftharpoons (RCO_2H)_2 + (R^1CO_2H)_2 + (RCO_2H)(R^1CO_2H)$$

Scheme 12

from the equilibrium the others will disproportionate so that eventually the substrate and reagent can all be consumed and the formation of substrate dimers or reagent dimers is an irrelevancy. For ease of analysis the substrate carboxylic acid selected was the hemisuccinate of a straight-chain alcohol so that we could use the same analytical method we have used previously for such functionalized alcohols.

When the mixture of benzophenone carboxylic acid and a straight-chain ester of succinic acid was irradiated in carbon tetrachloride and the product was then hydrolysed, dehydrated, and ozonized, a mixture of keto-alcohols was produced (Scheme 13). This reflected co-ordination of reagent with substrate, attack by the benzophenone carbonyl on substrate hydrogens, and collapse of the resulting complexed diradical to form a new carbon–carbon bond. The distribution in this product (Figure 7), determined by the thioketal method, was again consistent with our previous picture involving these rather long flexible substrates. There is high selectivity for attack in the accessible region of the chain, but a distribution over the accessible carbons.



Scheme 13

When the same process was applied to a steroid, androstan-3α-ol, models indicated that the benzophenone carbonyl should attack far out on the D ring. Indeed, attack and coupling occurred principally at C-16 (Scheme 14). Dehydration and oxidation afforded 16-ketoandrostanol as the only steroidal ketone, although some other materials remain unaccounted for in this process. Controls establish in this case, as in all the cases we have discussed, that under reaction

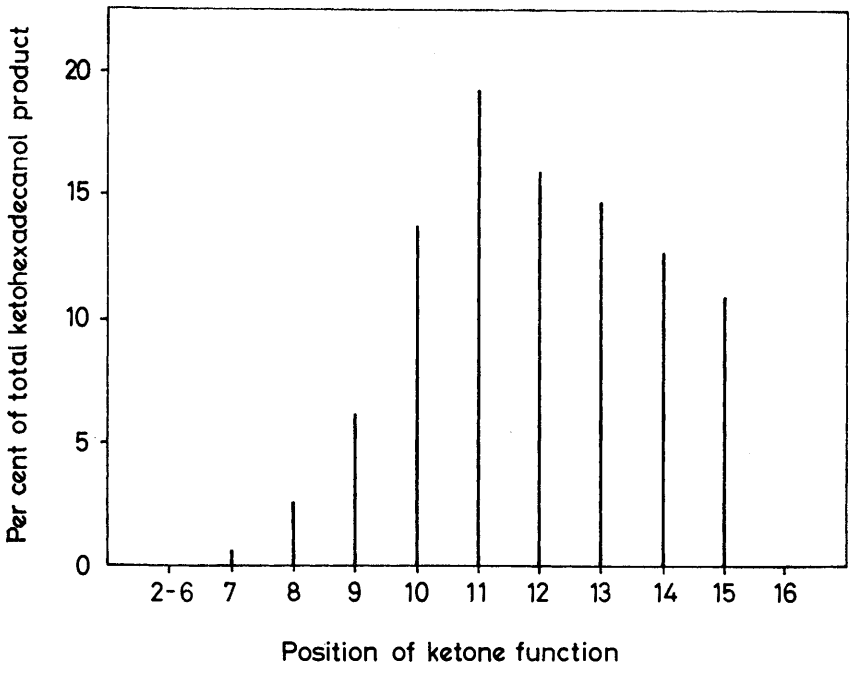
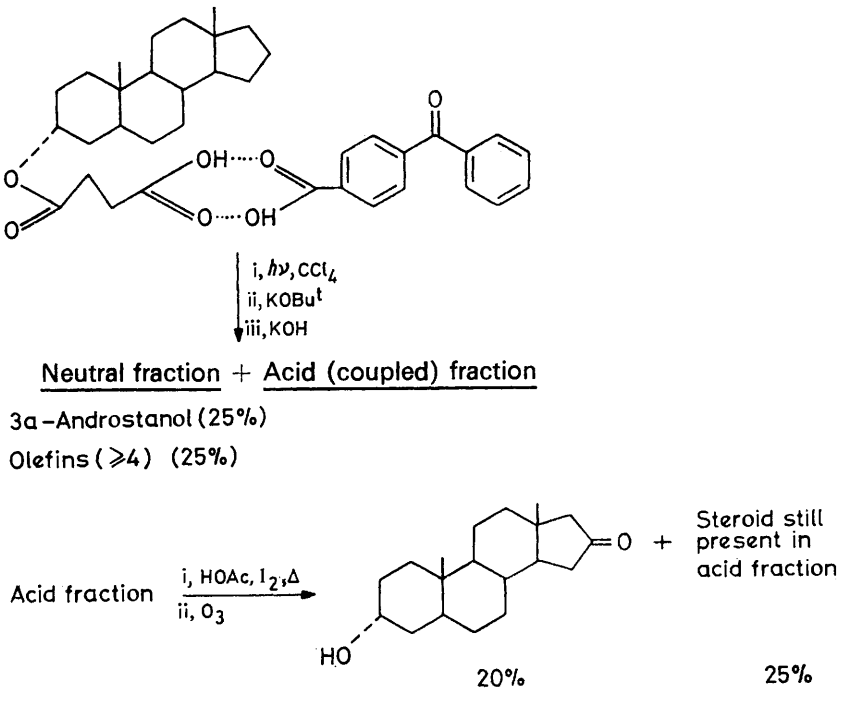


Figure 7 *Ketohexadecanol isomer distribution*

conditions, 10^{-3} mol l^{-1}, the intramolecular or intracomplex process is much faster than any intermolecular reactions. Thus, the selectivity is expected.

It is interesting that in this situation, involving hydrogen-bonded complexing, the product diradical is held together and can undergo coupling. In a control using a simple acetate ester of the steroid, so that no hydrogen-bonded complexing is possible for either step, the initial photochemistry is much slower. However, if it is allowed to proceed to completion there is still no detectable product from coupling of reagent with substrate. Thus, the hydrogen bonding is involved in directing the initial attack of the reagent, and giving it a fast rate by producing a high local concentration of the reagent. It is also involved in holding the intermediate radical pair together, so that coupling can occur and lead to the overall functionalization described. Our studies on other kinds of complexing are still under way.

In some studies on cyclodextrins (cycloamyloses) we have also managed to achieve biomimetic functionalization by orientation of reagent and substrate in a well-defined geometry, with attack on a particular spot within the reagent–substrate complex. The cyclodextrins have the interesting property that they

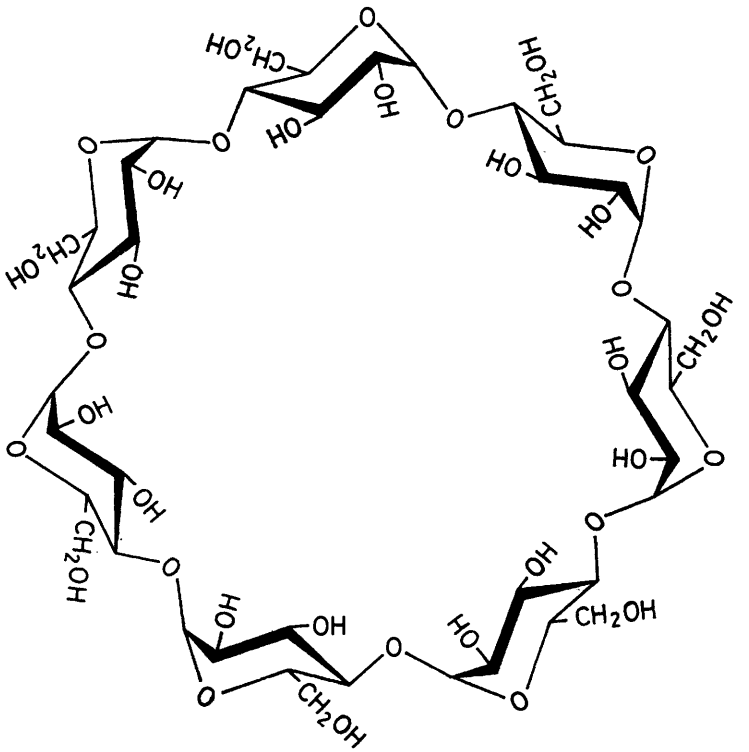


Scheme 14

are water soluble, but have a hydrophobic cavity. Thus, in aqueous solution hydrocarbons or hydrocarbon groups of other molecules will be bound into this cavity for the usual hydrophobic reasons. In particular, α-cyclodextrin (cyclohexa-amylose) binds simple benzene derivatives quite well. β-Cyclodextrin (cycloheptа-amylose) (3) has a larger cavity which leads to somewhat more flexibility in the complex and different binding constants.

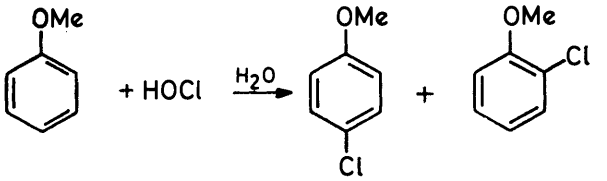
It occurred to us that in the cyclodextrin complex of an aromatic ring, such as that of anisole, the *ortho* positions on the ring would ordinarily be buried in the cavity. The *para* position of the ring might be accessible, either to free solution or to the hydroxyls which rim the cavity. Thus, it might be possible to achieve a selective aromatic substitution on the *para* position in such a case, and suppress the *ortho* substitution which is a common accompaniment of electrophilic aromatic substitution processes.

The reaction selected for study in detail is the chlorination of anisole with HOCl (Scheme 15). It can be performed in water solution, which is necessary



(3)

for the hydrophobic bonding, and gives a fairly random distribution of *para*- and *ortho*-chloroanisole in the absence of our cyclodextrin reagent. As Table 4 indicates, the normal substitution pattern, 60% *para* and 40% *ortho* chlorination, is modified to an almost exclusively *para* chlorination process in the presence of 10^{-2} M cyclohexa-amylose. In fact, the new ratio of 96% *para* and 4% *ortho* chlorination occurs under conditions in which only 72% of the anisole is bound



Scheme 15

in the cavity. A number of techniques are available[11] to determine such binding constants.

Table 4

Cyclohexa-amylose/ 10^3 mol l^{-1}	*Chloroanisole product ratio*, p:o	% *anisole bound*
0	1.48	0
0.933	3.43	20
1.686	5.49	33
2.80	7.42	43
4.68	11.3	56
6.56	15.4	64
9.39	21.6	72

These data indicate that the anisole is actually more reactive inside the cavity than outside, and that the cyclodextrin not only suppresses *ortho* chlorination but also facilitiates *para* chlorination. By contrast, other sugars have no effect on this ratio, and even cyclohepta-amylose changes the selectivity to a much lesser extent. As the data in Table 5 indicate, the anisole is slightly less well bound in this larger cavity, and it obviously is also more flexibly bound in the cavity.

Table 5 *Chlorination of anisole by* HOCl

Cyclohepta-amylose/ 10^3 mol l^{-1}	p:o *product ratio*	% *anisole bound*
0	1.48	0
0.944	1.69	12
1.752	1.88	20
2.98	2.16	29
5.07	2.63	41
7.05	3.08	50
10.12	3.77	59

These data can be analysed in terms of a partial rate factor treatment, but it is necessary in this case to define the concentration of hypochlorous acid at which the 'partial rate factors' are specified. Table 6 indicates that taking the *para* position of free anisole as the standard, an *ortho* position in free anisole is one-third as reactive. This is simply a translation of the 60% *para*, 40% *ortho* chlorination findings incorporating the fact that there are two *ortho* positions. However, in the complexed anisole in either cavity the *ortho* positions are inert.

[11] R. Breslow and P. Campbell, *J. Amer. Chem. Soc.*, 1969, **91**, 3085; *Bioorg. Chem.*, 1971, **1**, 140.

Table 6 *Partial rate factors for anisole chlorination* (HOCl = 10^{-2} mol l^{-1})

	k_{free}	k_{bound}	
		cyclohexa-amylose	*cyclohepta-amylose*
para	1.000	5.31 ± .02	1.1 ± .05
ortho	0.34 ± .02	0.01 ± .05	−0.02 ± .05

$$\text{rate (free)} = k_{free}\,[\text{anisole}]\,[\text{HOCl}]^2$$
$$\text{rate (bound)} = k_{bound}\,[\text{anisole complex}]\,[\text{HOCl}]^1$$

Thus, it is not possible to attack the buried *ortho* positions in the complex. The cyclohexa-amylose also catalyses *para* chlorination by a factor of 5.3 under these conditions, while the larger cyclohepta-amylose gives essentially the same rate for the *para* chlorination inside and outside the cavity.

The most striking observation is that the kinetics are different in the case of the complex from those for free anisole. With free anisole the chlorination is second-order in HOCl; the true chlorinating species is Cl_2O, which is in equilibrium with two HOCl molecules. However, with the cyclodextrin complex the kinetics involve only the first power of HOCl, indicating that the chlorinating agent in this case is a species with a single chlorine atom.

All of this is most consistent with the mechanism shown in Figure 8, in which

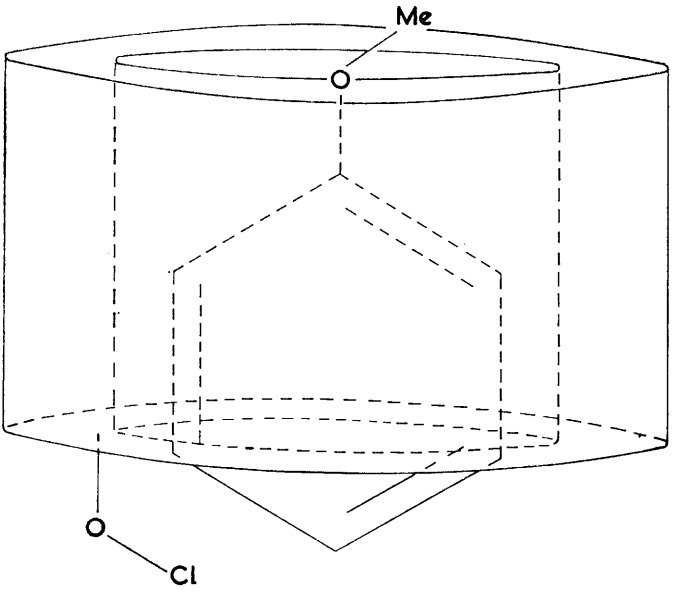
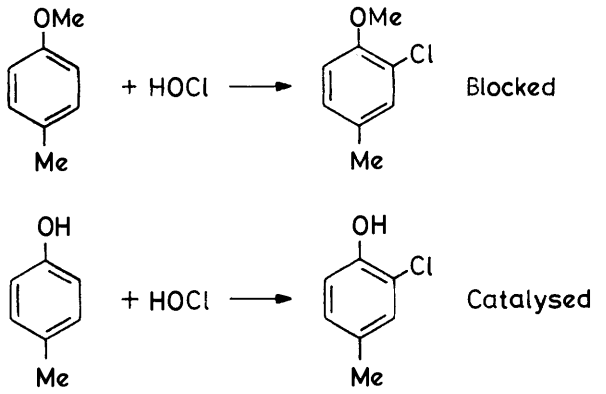


Figure 8 *Schematic representation of an anisole molecule in the cavity of cyclohexa-amylose. Eighteen hydroxy-groups (not shown) ring the mouths of the cavity, one of which is written as its hypochlorite ester to indicate a mechanism by which the increased rate of chlorination in the complex may be explained*

anisole is bound in the cavity and a chlorine is delivered not from free solution but from one of the neighbouring hydroxyls. With hypochlorous acid alcohols are in rapid equilibrium with hypochlorite esters. The neighbouring group effect explains why a species with a single chlorine atom is reactive enough to perform the halogenation. The complex with the larger β-cyclodextrin cavity is slower, since flexibility in this complex decreases the advantage of a neighbouring group.

Additional interesting evidence comes from the study of *p*-cresol derivatives. Thus (Scheme 16), in the methyl ether of *p*-cresol the position *para* to the methoxyl is, of course, no longer available. Hypochlorous acid produces *ortho* chlorination with or without the cyclodextrin, but the complex is inert and cyclodextrin slows the reaction. In the case of *p*-cresol itself, hypochlorous acid also produces the *ortho* chlorination but now this is *catalysed* by α-cyclodextrin. It seems that the geometry of the *p*-cresol complex is such that the toluene section of the substrate is buried in the cavity, but the hydroxy-group protrudes into the hydroxylic solvent. Under these circumstances, the *ortho* position is accessible to the neighbouring hydroxyls, and chlorine can be directly delivered to these positions.

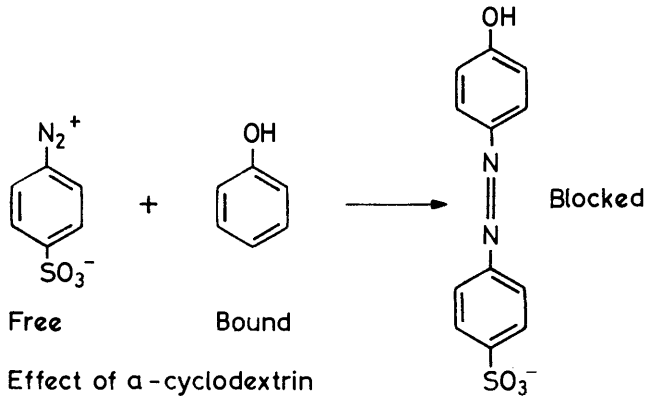


Scheme 16

An additional piece of evidence for our mechanism is that the diazo coupling of phenol with an unbound diazonium salt is blocked by α-cyclodextrin (Scheme 17). When the phenol is in the complex it is not accessible to attack by reagents from free solution. Only reagents which can be delivered from the hydroxyls rimming the cavity undergo catalysed substitution reactions.

A final interesting point about our process is that there is an enzyme, chlorinase, which will also chlorinate anisole.[12] We might inquire as to how selective our

[12] F. S. Brown and L. P. Hager, *J. Amer. Chem. Soc.*, 1967, **89**, 719.



Scheme 17

simple biomimetic reaction is compared with the action of this enzyme. It is an interesting fact that ours is the more selective process, since chlorinase gives essentially the same 60:40 ratio for chlorination of anisole which is observed with HOCl. The enzyme is apparently generating a chlorinating agent which attacks anisole, and anisole itself may not be bound to the enzyme. Thus this particular enzymic process does not have typical enzymatic selectivity.

Even in their present very primitive state biomimetic reactions have some advantages over enzymatic processes. Thus they can be applied to achieve selective reactions for which no natural enzymes exist. In the case of an enzyme such as chlorinase we can achieve greater selectivity in chlorination of anisole with a biomimetic reaction because the natural reaction has not been optimized for this selectivity. Biomimetic chemistry does not yet have the high selectivity of the well-optimized enzymatic reactions, but the selectivities already achieved suggest that biomimetic reactions have an important role to play in the synthetic methods of the future.[13]

[13] Support of this work by the National Institutes of Health is gratefully acknowledged.