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STEREOSPECIFICITY OF SOME ENZYMIC REACTIONS

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One cannot help but be amazed by enzymes. They are astonishingly efficient as catalysts, they are often highly selective with respect to the molecule(s) they will accept and the reactions they catalyse are precisely controlled. One important manifestation of this control is *stereospecificity*. Organic chemists often in collaboration with biochemists or enzymologists have been working out in precise stereochemical terms what happens when one intermediate on a biochemical pathway is converted into the next one. Such knowledge is available now for many such conversions and this information is one essential contribution to be added to many others from different approaches which in the long run should allow a complete description of an enzymic reaction. The plan in this survey is to outline a variety of studies carried out by several research groups so as to bring out the beauty of Nature's stereochemical work and also we hope, to show what enjoyment there is here for organic chemists.

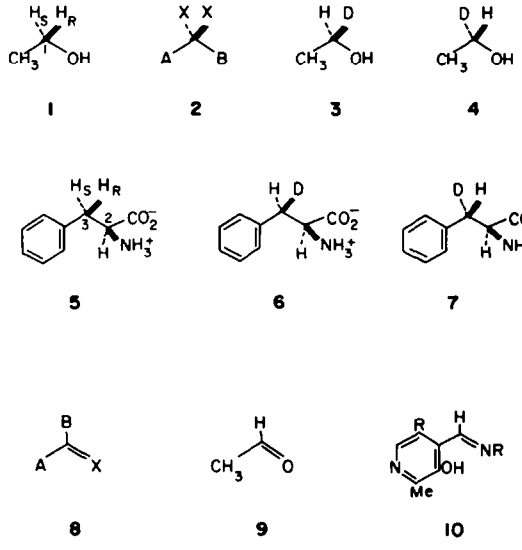
Much equally important work will understandably not be covered for all we can hope to do in this short outline is to illustrate the approaches.

This relatively recent progress falls best into place when it is viewed against the historical background. The story of Pasteur's discovery of stereochemical specificity in the attack by living moulds on suitable organic substances is one of the most fascinating in our science.[1] These aspects and the subsequent development of ideas about active sites and stereochemical specificity of enzymes including Ogston's hypothesis and its modern equivalent are described by Professor J. W. Cornforth in the historical introduction to his paper for this volume. Accordingly we can turn directly to a fascinating and subtle aspect of enzyme stereochemistry, namely, reactions which involve formation or destruction of prochiral centres. A brief outline of this aspect of stereochemistry and an explanation of the terms which are used will no doubt be helpful to the general reader.

Prochirality[2]

A molecule such as ethanol (1) is said to be *prochiral* in that C-1 carries two chemically identical H atoms and two non-identical groups (Me and OH) which are not chiral; a simple, more general case is structure 2. The two H atoms of ethanol (and the X groups of 2) are called *enantiotopic*. Mental replacement of each C-1 H atom in turn by a third different group generates two *enantiomers* and this is a simple way to check whether two atoms or groups are enantiotopic or not. This replacement has been carried out for ethanol using deuterium to produce structures 3 and 4, which are clearly enantiomeric. Their respective configurations are *R* and *S*. That H atom in ethanol which on replacement by deuterium gave *R*-monodeuterioethanol is called the *pro-R* H atom and marked H_R (see 1). Similarly, the H atom which on replacement by deuterium generates *S*-monodeuterioethanol is designated the *pro-S* H atom and is denoted H_S.

So far, the groups A and B in structure 2 and the analogous groups in ethanol (1) have not been chiral. However, if say group B of structure 2 is chiral, then replacement of each X group in turn by a third different group will produce diastereoisomers. This is the case for (2*S*)-phenylalanine (5) where it can be seen that the diastereoisomers (2*S*, 3*R*)-[3-^2H_l]phenylalanine (6) and (2*S*, 3*S*)-[3-^2H_l]phenylalanine (7) are the products of replacement one at a time of the H atoms at C-3 in this case



1 2 3 4 5 6 7 8 9 10

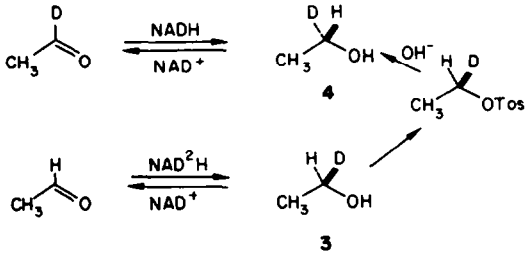
by deuterium. Accordingly, these C-3 H atoms (or groups in the general case) are termed *diastereotopic* atoms or groups. The same system described above for the simpler case of ethanol is used to designate the *pro*-R and *pro*-S H atoms at the prochiral centre (see **5**).

There is a further type of prochiral system which is of great importance for the later discussion. Though it was initially by no means as obvious as it now appears, the two faces of the molecule (**8**) are distinguishable by a chiral reagent, *e.g.* an enzyme which is certainly that, *par excellence*. The system of notation for the two faces involves viewing the molecule (**8**) from above and setting the groups A, B and X in sequence according to the Cahn–Ingold–Prelog rules. If the order of descending priority is clockwise, then the upper face is designated *re*; conversely, if it is anticlockwise the term *si* is used (*re* = rectus and *si* = sinister). For acetaldehyde **9** the *re*-face is the upper one as it is written and similarly for the imine (**10**) derived from pyridoxal.

Chirally labelled substrates

Many of the explorations of enzymic stereospecificity which will be outlined in the sequel depend upon the preparation of substrates which are chirally labelled at the centre of interest. Both chemical and enzymic methods have been used (or the two in combination) to produce these labelled materials. The classical researches which were carried out on alcohol dehydrogenase and its coenzyme not only illustrate beautifully the fundamental approaches but can be drawn upon for other examples later.

Westheimer, Vennesland and their colleagues[3] found that when [1-$^2H_1$] acetaldehyde is reduced by yeast alcohol dehydrogenase and its coenzyme (reduced nicotinamide adenine dinucleotide, NADH) a sample of [1-$^2H_1$] ethanol was formed which will later be shown to be the *S*-isomer (**4**). This sample was oxidised again by the enzyme and the oxidised form of its coenzyme (NAD^+) to give [1-$^2H_1$] acetaldehyde without loss of deuterium. A complementary set of experiments also gave beautifully clear results in that use of reduced deuteriated coenzyme (NAD^2H) with unlabelled acetaldehyde in the enzymic reaction led to a second sample of [1-$^2H_1$] ethanol which on reoxidation now lost all its deuterium; this second sample will be shown to be the *R*-isomer (**3**). It was clear at this stage, without having knowledge of the absolute configurations of the chirally labelled ethanols that a highly stereospecific process was being observed and the evidence became overwhelming when the ethanol (**3**) was inverted *via* its tosylate as illustrated in Scheme 1 and the product (**4**) was then found to lose hydrogen only in the enzymic reoxidation to acetaldehyde.



SCHEME 1.

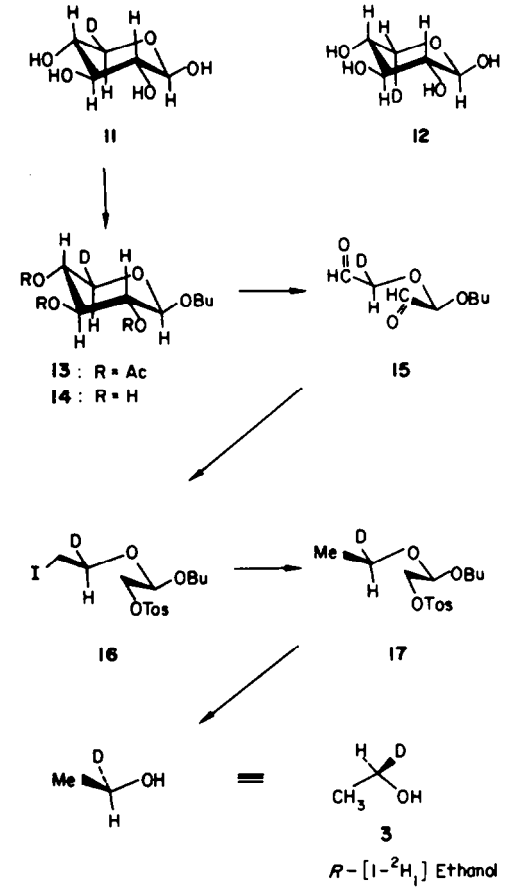
These experiments, when run on a larger scale,[3] also gave the opportunity to find out in the case of a simple alcohol whether chirality arising from isotopic substitution could give rise to measurable optical activity.[4] It was found that for [1-$^2H_1$] ethanol, a small rotation could readily be observed.

Two stereochemical problems now cried out for solution. One was the question of absolute configuration of the two [1-$^2H_1$] ethanols considered above and the other was the complementary stereochemical problem for the nicotinamide coenzyme. We can deal first with the former puzzle which attracted several groups of workers[5-8] and four solutions have been provided, all satisfyingly in agreement.

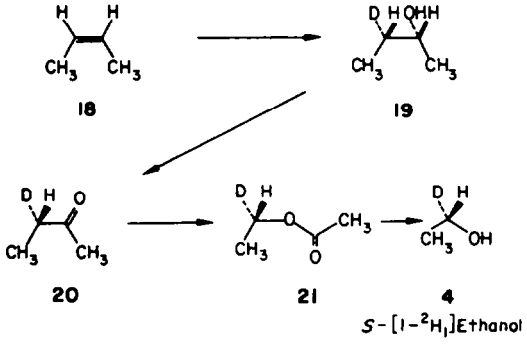
An outline of two will show the method of attack. Lemieux and Howard[5] set up the required chirality on a carbohydrate framework by synthesising the deuteriated β-D-xylose from glucose by a sequence which need not concern us. The [5-$^2H_1$] xylose [(**11** in Scheme 2)] was not configurationally pure, there being *ca* 65% of it in the product and the rest was the epimeric product (**12**). However, we will see here and for several examples later that problems can often be definitively solved with substrates of less than 100% configurational purity. The reaction sequence will be illustrated (Scheme 2) only for the major isomer but obviously the admixed minor one (**12**) was carried forward with it throughout.

Conversion of the [5-$^2H_1$]xylose via its tri-O-acetyl-1-bromo derivative first into the protected ether (**13**) and then into the deprotected one (**14**) was followed by periodate cleavage. This opened the molecule to allow the deuterium-carrying carbon to be prized out from the dialdehyde (**15**). Borohydride reduced both carbonyl groups, the resultant alcoholic functions were tosylated and the product was selectively attacked by iodide anion. Reduction of the mono-iodide (**16**) then gave the desired ethanol derivative (**17**) in a readily cleavable form. The [1-$^2H_1$]ethanol thus cunningly obtained showed a positive rotation and since the predominant product from this reaction sequence must have the *R*-configuration the *sign of rotation* and the *absolute configuration* had been connected.

Arigoni's group[6] used an attractive different correlation (Scheme 3). Asymmetric hydroboration of *cis*-2-butene (**18**) with chiral deuteriated tetra - 3 -



SCHEME 2.

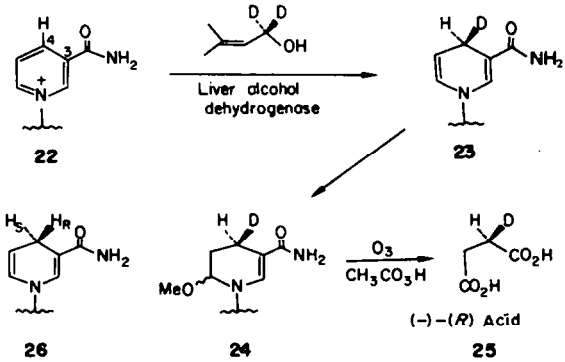


SCHEME 3.

pinanyldiborane occurred by addition which was shown to be experimentally indistinguishable from 100% *cis*. The extent and chiral sense of the asymmetric induction could be assayed by the optical rotation of the product (**19**) in comparison with standard configurationally pure 2-butanol. Notice that the chiral centres at C-2 and C-3 have been configurationally linked by the method used for their generation. Structure **19** is thus secure as an absolute representation of the major deuteriated alcohol in the mixture. The rest of the illustrated sequence involving catalytic oxidation to yield **20** and Baeyer–Villiger rearrangement to the ester **21** shows a careful selection of reactions and conditions to avoid significant racemisation of the chiral deuteriated centre. The final ethanol (which by its synthesis must contain predominantly the *S*-isomer) was correlated in this case directly with the enzymic process by proving that it largely *retained* its deuterium when yeast alcohol dehydrogenase and NAD^+ oxidized it to acetaldehyde.

The stereochemical problem of the reduced nicotinamide coenzymes† was solved by Cornforth, Popjak and their colleagues[9] who reduced unlabelled NAD^+ (**22**) using liver alcohol dehydrogenase with the illustrated deuteriated alcohol (Scheme 4). Chemical degradation of the labelled reduced coenzyme (**23**) by protonation of the more electron-rich double bond and concomitant addition of methanol to give **24** was followed by ozonolysis; oxidative work up then afforded monodeuteriosuccinic acid. The monodeuteriosuccinic acids have proved to be important primary standards in this area in that accurate measurement of their specific rotation can be made on milligram scale with a suitably sensitive instrument (see p. 1710 for the determination of their absolute configuration). The sample obtained from Scheme 4 was shown to have the *R*-configuration (**25**). Similarly, (*S*)-monodeuteriosuccinic acid was obtained from a complementary sequence in which the labelled reduced coenzyme was obtained by enzymic reduction of [4-²H]NAD^+ (*cf* **22**) with normal (unlabelled) alcohol.

It follows from this work that enzymic transfer of hydrogen occurs onto the upper face of NAD^+ as it is written in Scheme 4 (the *re*-face); in the reverse reaction, it is H_R of NADH (**26**) which is carried to



SCHEME 4.

†The oxidized form is NAD^+ (part structure **22**) and the reduced form is NADH (part structure **26**).

the substrate. This stereospecificity holds good for both liver and yeast alcohol dehydrogenases but there are others (e.g. glucose 6-phosphate dehydrogenase from yeast) which show the opposite stereospecificity in the $NAD^+ \rightleftharpoons NADH$ redox process.

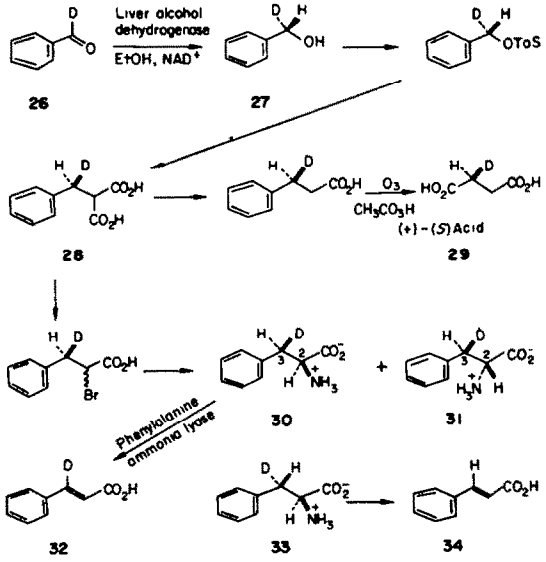
Stereochemistry of enzymic reactions involving attack at benzylic centres

A large variety of biochemical transformations involve the formation or destruction of prochiral centres adjacent to an aromatic nucleus; this is particularly evident in the chemistry carried out by higher plants. One example is the formation of cinnamic acid (**34**) from L-phenylalanine (unlabelled **33**) catalysed by the enzyme phenylalanine ammonia lyase.[10] This conversion is an important early step in the biosynthesis of lignin and it is interesting to think of the vast scale of this process in the forests of the world. Our studies of this and other enzymic reactions at benzylic centres required the synthesis of chirally labelled substrates from chirally labelled benzyl alcohols. A "coupled" reducing system (**26**→**27**, Scheme 5) provided (+) - (*S*) - [*methylene* - $^2H_1$]benzyl alcohol (**27**) in gram quantities and the corresponding tritiated alcohol was also readily prepared by this method. There was earlier evidence[11] that (+)-monodeuteriobenzyl alcohol has the *S*-configuration. The synthetic sequence and degradation[12] shown in Scheme 5 confirm this assignment by correlation of key intermediate **28** with (+) - (*S*) - monodeuteriosuccinic acid (**29**). Scheme 5 also shows how (2*S*, 3*R*) - [3 - $^2H_1$]-phenylalanine (**30**) and the (2*R*, 3*R*)-isomer (**31**) were prepared. Since phenylalanine ammonia lyase acts only on the L-form (2*S*-isomer), the mixture of **30** and **31** could be used to study the stereochemistry of the elimination process. The cinnamic acid (**32**) formed enzymically contained all the deuterium of the starting amino acid (**30**). Conversely, (2*S*, 3*S*) - [3 - $^2H_1$]phenylalanine (**33**), prepared by a different route,[12] lost its deuterium when converted into cinnamic acid (**34**) by phenylalanine ammonia lyase.

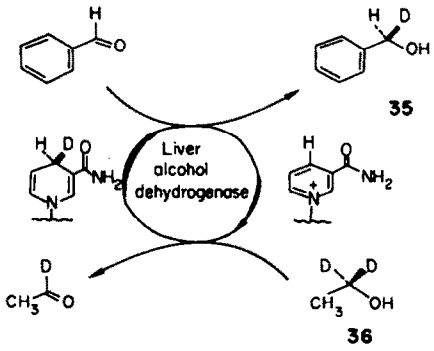
This rigorously defined stereochemistry for the enzymic elimination process is consistent with a mechanism in which the 3-*pro*-*S* H atom of L-phenylalanine (**5**) and the product of the reaction between the substrate's amino group and the enzyme's prosthetic group[10] are eliminated in an antiperiplanar manner to generate *trans*-cinnamic acid.

In the foregoing research, the two chirally labelled samples of phenylalanine were prepared by different methods and no ambiguities arose because both procedures yielded products of quite high configurational purity. Thus, the route illustrated in Scheme 5 afforded **30** and **31** which were 95 ± 5% configurationally pure at C-3. However, appreciable racemisation can sometimes occur in some synthetic sequences so that the configurational purity of the final product may lie in the range 70–80% (i.e. 70–80% of say the *R*-configuration at the centre of interest in admixture with 30–20% of material with the *S*-configuration at that centre). In this situation, it is important to arrange the synthesis so that *both* the *R*-, and *S*-substrates can be studied in the enzymic reaction and further, they should be synthesised by a route which generates strictly complementary products. By this we mean that if the *R*-isomer is in fact 80% *R* and 20% *S*, then it should be arranged that the product which is largely *S* contains 80% *S* and 20% *R*. If this precaution is taken, then rigorous, unambiguous results can be obtained without there being the slightest disadvantage arising from the less - than - perfect configurational purity.

One way to achieve this complementarity would



SCHEME 5.



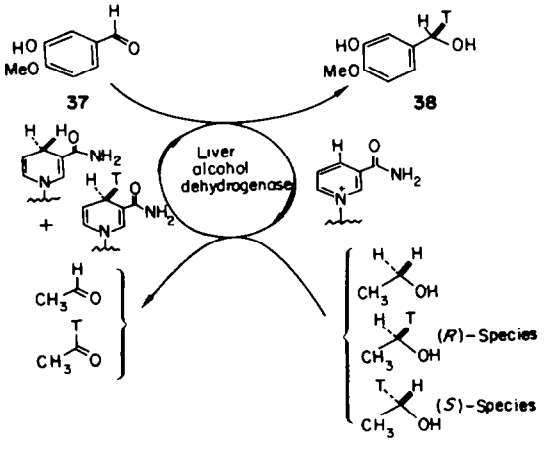
SCHEME 6.

be to prepare the enantiomeric labelled benzyl alcohols (e.g. **27** and enantiomer) and then to carry each one through the synthetic sequence under strictly parallel conditions. In principle this appears simple and one would plan to synthesise one chirally labelled benzyl alcohol as in Scheme 5 by enzymic reduction of deuterium or tritium labelled benzaldehyde. The enantiomer might then be obtained by enzymic transfer of deuterium or tritium to unlabelled benzaldehyde from ethanol labelled at C-2. This is readily carried out with deuterium but considerable difficulties, not immediately obvious, had to be overcome[13] before this was possible for tritium. A discussion of the problems involved will be instructive in bringing out the different approach necessary for experimental work with these two hydrogen isotopes.

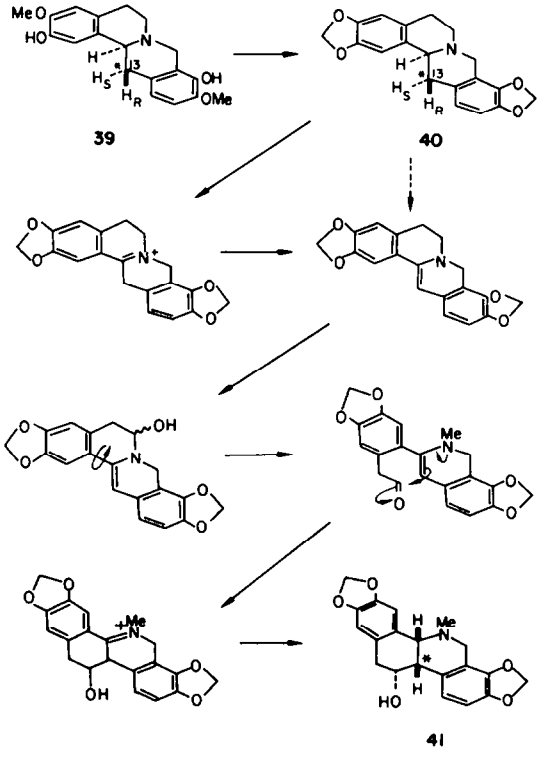
[1-$^2H_2$]Ethanol (**36**) is readily prepared with a high isotope content (over 99%) and can be used in the coupled system illustrated in Scheme 6 to reduce benzaldehyde to (*R*) - [*methylene* - $^2H_1$]benzyl alcohol (**35**). The reduction is slower than when normal ethanol is used because of the kinetic isotope effect in rupture of the C–D bond of the deuterio ethanol (**36**). However, this is only a minor problem unless you have allowed before dinner exactly the same time to run the reaction which you have used many times in the normal (protium) series; if so, you then *experience* an isotope effect.

The tritium series is quite different. Here the isotope is at tracer level which means that the vast majority of molecules in [1-$^3H_1$]ethanol contain no tritium. The labelled molecules carry only one isotopic H atom so that *R*-, and *S*-species are present as a 50:50 mixture (Scheme 7). It will be evident from our earlier discussion of the stereospecificity of liver alcohol dehydrogenase that only half of the valuable labelled molecules can transfer tritium (from the *R*-species). Even more crippling is the kinetic isotope effect. Those ethanol molecules which can react by rupture of a C–H bond will do so faster than those which would involve C–T cleavage. So there is discrimination against transfer of tritium in this step. A similar discrimination occurs again in the next hydrogen transfer from the mixture of tritiated and unlabelled NADH which has been formed (Scheme 7). Reduction thus runs markedly ahead of tritium transfer and we have made an approximate experimental determination of the combined kinetic isotope effect, that is, for transfer onto and off the coenzyme. This gave a value of $K_H/K_T = 14$ for the combined process.

Our colleague Dr. H. R. Wiltshire[13] overcame these problems by combining a good idea with a careful study of the reduction process (pH, equilibria, rate of ^1H and ^3H transfer etc). The idea, simple but effective, was to use a secondary alcohol as the reducing agent in place of tritiated ethanol and *cyclo*hexanol was chosen; it was prepared by reducing *cyclo*hexanone with borotritiide. All the tritium of [1-^{3}H]*cyclo*hexanol is available for transfer. Knowing the various factors controlling the coupled reactions, it was then possible to devise conditions which allowed 97% of the tritium in [1-^{3}H]*cyclo*hexanol to be transferred to the substituted benzaldehyde (**37**) so forming alcohol (**38**). O-Benzylation then afforded the protected (*R*)-alcohol (**42**). The enantiomer of **42** was readily pre-



SCHEME 7.



SCHEME 8.

pared by enzymic reduction of the [*formyl*-^{3}H]aldehyde (tritiated analogue of 0-benzyl ether of **37**) in the coupled system using unlabelled ethanol as the reducing agent. In this way, complementary samples of the *R*-, and *S*-tritiated benzyl alcohols (**42** and enantiomer) were available for chemical conversion into the required precursors.

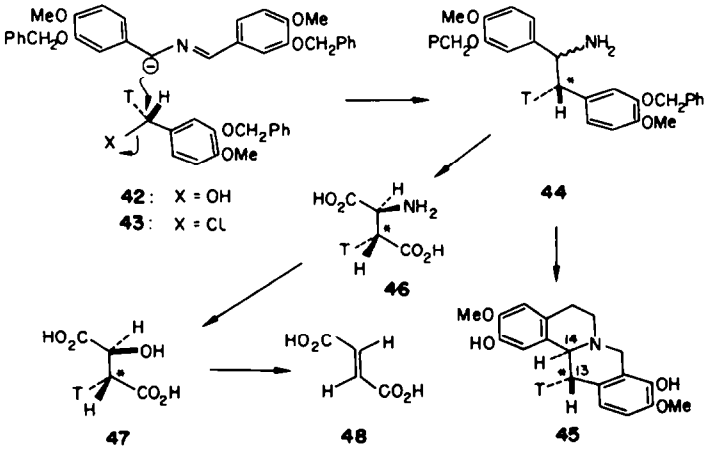
These tritiated materials were needed for studies of two skeletal conversions of particular interest which occur in certain higher plants. The first is found in the biosynthesis of chelidonine (**41**), the major alkaloid of the greater celendine, *Chelidonium majus*. Earlier studies[14] of this natural product had shown that it is formed from (−)-scoulerine (**39**) with the next step being conversion into (−)-stylopine (**40**). Dehydrogenation, ring-cleavage, N-methylation, C–C bond formation and reduction then follow, the sequence being unknown at present; a chemically plausible order has been illustrated in Scheme 8. We will concentrate on what happens at the prochiral benzylic carbon at C-13 of scoulerine (**39**) and stylopine (**40**) because this is a centre of mechanistic importance not only for this case but also for the second example to be discussed in the sequel.

The problem at this stage is one of converting the tritiated *R*-, and *S*-alcohols (**42** and enantiomer) without serious racemisation into scoulerine; by using strictly the same reaction sequence and conditions, the two final products will be labelled in a stereospecific and complementary way at position-13. A few key steps in the synthesis of (13*S*) - [13 - ^{3}H$_1$]scoulerine (**45**) are shown in Scheme 9 and two important points about this sequence need to be emphasised. A good sequence builds the labelled chiral centre as quickly as possible into a site where it is safe from racemisation. Having achieved this, the configurational purity of the labelled intermediate should then be determined. It is not possible here to give a full account of the rigorous determination which was made in this case. However, in brief, it involved ozonolysis of the amine (**44**) to yield DL-aspartic acid which was resolved and the L-form (**46**) was converted into L-malic acid (**47**) which was assayed with the enzyme fumarase. Fumarase is known[15] to remove the pro-*R*-H atom from the asterisked carbon of L-malic acid so the configurational purity of the original amine (**44**) is readily calculated from the tritium lost in the formation of fumaric acid. Here we are using the increasingly common possibility of making one enzyme do some work in studying another enzymic reaction.

The amine (**44**) which was to yield (13*S*) - [13 - ^{3}H$_1$]scoulerine† (**45**) was shown in this way to contain 75 ± 4% of (13*S*)-species, the remainder being the (13*R*)-amine. Conversely, the amine (as **44**, epimeric at C-13) which was to yield (13*R*) - [13 - ^{3}H$_1$]scoulerine was found to comprise 73 ± 4% of (13*R*)-species with the remainder being (13*S*)-amine. So the expected complementarity was confirmed and the configurational purity was quite high enough to give definitive results in the enzymic studies.

Completion of the synthetic sequences through to the two labelled forms of (−)-scoulerine (**45** and its epimer at C-13) allowed incorporation experiments to be carried out on living *C. majus* plants. [^{14}C]Scoulerine was mixed with the tritiated precursors to act as an internal standard; the retention or loss of tritium can then be determined by measuring the ^{3}H:^{14}C ratio in the precursor and product. The results were highly satisfying. Plants fed with (13*S*)-labelled scoulerine (**45**) yielded chelidonine

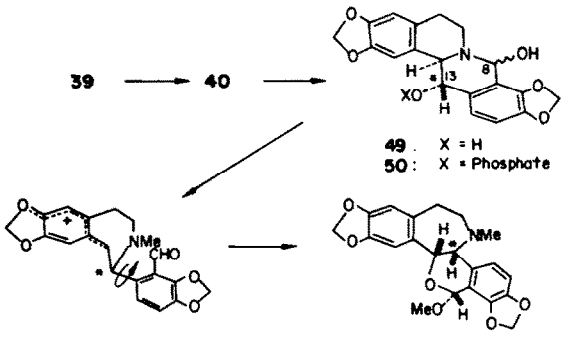
†The synthetic product (as **45**) is of course racemic at C-14 but only the (−)-scoulerine (illustrated in Schemes 8 and 9) acts as a precursor of chelidonine (**41**) in the living system. The (+)-isomer is inactive and can be ignored.



SCHEME 9.

which had *lost* 72% of the original tritium whereas the (13*R*)-precursor gave chelidonine with tritium *retention* of 75% (*cf* the configurational purities of the starting materials given in previous paragraph). These results demonstrate that in the conversion of the benzylic carbon atom at C-13 of (−)-scoulerine (**39**) and (−)-stylopine (**40**) into the asterisked carbon of chelidonine (**41**), there is a cleanly stereospecific removal of the *pro-S* H atom (i.e. the rear one as drawn).

This result should be mentally carried forward for comparison with that found for a different skeletal rearrangement to be discussed now which is characteristic of *Papaver rhoeas* (the Flanders poppy). This plant constructs (+)-rhoeadine[16] (**51**) and it has recently been shown by tracer experiments[17] that rhoeadine, like chelidonine (**41**), arises by extensive modification of scoulerine (**39**). Little is known at present about the nature of the rearrangement process but clues should be forthcoming by studying the stereochemistry of changes at key centres. The benzylic carbon at position-13 of scoulerine (**39**) is one of these.



SCHEME 10.

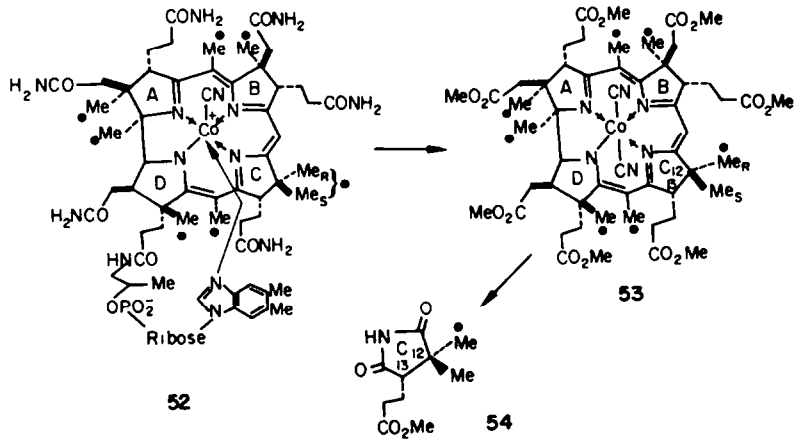
The (13*S*)-, and (13*R*)-labelled scoulerines (**45** and epimer at C-13) which were used for the study of chelidonine were put into service here also. Both were incorporated by living *Papaver rhoeas* plants into rhoeadine (**51**) and clear cut $^{3}H:^{14}C$ ratios were again found in the isolated alkaloid. The rhoeadine isolated from plants fed with (13*S*)-labelled scoulerine (**45**) had *lost* 79% of the tritium present in the precursor whereas the (13*R*)-labelled scoulerine (**45**, epimeric at C-13) afforded rhoeadine which *retained* 74% of the original tritium. Bearing in mind the configurational purity of the precursors (see earlier), these values prove that a stereospecific loss of the *pro-S*-hydrogen occurs from C-13 of scoulerine (**39**) at some stage of its transformation into rhoeadine (**51**). It seems likely from structural considerations that as before, scoulerine† (**39**) is converted first into stylopine (**40**). Enzymic hydroxylation at C-13 from the rear side with retention of configuration[18] would remove the *pro-S*-hydrogen to set up the chirality (**49**) for rearrangement possibly *via* the phosphate (**50**); hydroxylation at C-8 is also envisaged. Rearrangement and N-methylation (not necessarily in this order) then provides the correct skeleton from which rhoeadine (**51**) could arise by obvious steps. It would be foolish in these early days to do more than sketch a possible pathway; nevertheless, we want to emphasise the power of the stereochemical approach in helping to pick out one pathway from many possibilities.

Stereochemistry of enzymic C-methylation in corrin biosynthesis

The examples we have considered so far have involved enzymic attack at a prochiral centre with loss of one of the two enantiotopic or diastereotopic H atoms present. We turn now to the reverse type of process in which a prochiral centre is *generated* and in the example chosen, diastereotopic methyl groups are set up by an enzymic *C*-methylation reaction. It was known[19] that one of the two *C*-methyl groups at position − 12 of vitamin B_{12} (**52**) arises by decarboxylation of an acetic acid residue whereas the other is inserted from S-adenosylmethionine. Three groups[20–22] fed [*methyl* - ^{13}C]methionine to *Propionibacterium shermanii* which produces vitamin B_{12} and by ^{13}C-NMR spectroscopy, all agree that *seven C*-methyl groups are methionine-derived comprising the six marked ● and one of the two methyls at C-12. It was important to discover the stereochemistry of the *C*-methylation process not only because of its intrinsic value but also to provide clues about the sequence over the late stages in corrin biosynthesis.

Our approach[20] was to convert the ^{13}C-labelled vitamin B_{12} (**52**) into heptamethylcobyrinate[23] (**53**) which was degraded by ozonolysis[24] to yield the ^{13}C-labelled ring C imide (**54**). This contained 14 ± 2 atom percent of ^{13}C. Assignment of the clearly separated ^{1}H-NMR signals from the two *C*-methyl groups of an unlabelled sample of this imide was already secure from the synthetic work of Eschenmoser and his colleagues.[25] When the ^{1}H-NMR spectrum of the ^{13}C-labelled imide (**54**) was measured, it showed two satellite signals (from ^{13}C–^{1}H coupling, J, 128 Hz) centred on the signal of reduced intensity from the *pro-R* methyl group at C-12. It follows that it is the *pro-R* methyl which is enriched with ^{13}C and is thus derived from methionine. This conclusion could only be in error if, during methanolysis to form the hepta ester (**53**), complete or at least major inversion had occurred at C-13 to give the 13-epi series[26] (epimer of **53** at

†The Schemes show the (−)-isomers of scoulerine (**39**) and stylopine (**40**) but this is tentative since it has not yet been proved that (−)-scoulerine (**39**), and not the (+)-enantiomer, is the precursor of rhoeadine (**51**).



SCHEME 11.

position-13) in the experiment involving the ^{13}C-labelled sample. This has now been rigorously excluded[27] and the foregoing work is unambiguous. There is thus an overall *trans C*-methylation process at ring-C as is evident from inspection for rings A, B and D.

Scott's assignment[21] of the labelled methyl group was by NMR based upon the γ-effect and is in agreement with ours but Shemin's group[22] reached the opposite conclusion. Essentially their work depends upon correlation of ^{13}C and ^{1}H resonances for vitamin B_{12} itself and it seems likely that the discrepancy is due to the literature assignments of ^{1}H signals from the many *C*-methyl groups being incorrect for those at C-12. The nine chiral centres around the corrin macrocycle of vitamin B_{12} provide rich opportunities for future stereochemical research on the biosynthesis of this fascinating substance.

This brief survey of enzyme mediated reactions will have been worthwhile if it has brought out the value and rigour of stereochemical studies in this area. The firm data provided by such studies are tests which any mechanistic proposals in the future must pass. Organic chemists can in this way make an important contribution to our understanding of enzymic processes; such researches make considerable demands both experimental and conceptual but are not without pleasure in return.

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