

# **Synthesis of Xanthoxal**

by

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## Declaration

I hereby certify that this research is a result of my own investigation which has not already been accepted in substance for any degree and is not being submitted in candidature for any other degree.

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## Abbreviations

<i>aq.</i>	aqueous
b.p.	boiling point
CCl <sub>4</sub>	carbon tetrachloride
CH <sub>2</sub> Cl <sub>2</sub>	dichloromethane
conc.	concentrated
d	doublet
dd	doublet of doublets
ddd	double doublet of doublets
DEPT	distortionless enhancement over polarization transfer
DIBAH	diisobutylaluminium hydride
EI	electron impact
equiv.	equivalent(s)
EtOAc	ethyl acetate
<i>fr.</i>	fraction
g	grams
H <sub>2</sub> SO <sub>4</sub>	sulphuric acid
HCl	hydrochloric acid
Hg	mercury
hrs.	hours
Hz	Hertz
lit.	literature
MgSO <sub>4</sub>	magnesium sulphate
MHz	mega hertz
min.	minutes
ml	millilitres
mm	millimetres
mmol	millimoles
mol	moles

N <sub>2</sub>	nitrogen
Na <sub>2</sub> SO <sub>4</sub>	sodium sulphate
NaCl	sodium chloride
NaHCO <sub>3</sub>	sodium hydrogen carbonate
NaOH	sodium hydroxide
NBS	<i>N</i> -bromosuccinimide
NH <sub>4</sub> Cl	ammonium chloride
NMR	nuclear magnetic resonance
q	quartet
r.t.	room temperature
s	singlet
sat.	saturated
soln.	solution
t	triplet
<i>t</i>	trans
<i>t</i> -BuOK	potassium <i>tert</i> -butoxide
<i>t</i> -BuOOH	<i>tert</i> -butyl hydroperoxide
THF	tetrahydrofuran
ZnCl <sub>2</sub>	zinc chloride

## Summary

The aim of this project was to synthesize xanthoxal, a natural product which is a possible abscisic acid precursor. The xanthoxal will serve as a vital link in a study on the biosynthesis of abscisic acid in plants. This study does not form part of this thesis.

The synthetic pathway begins with mesityl oxide and ethyl acetoacetate as starting materials, and the target molecule was obtained via a 13-step route. The first step involved a Robinson annulation, which afforded the ketal of a C<sub>10</sub>-ester ketone (ethyl-2,6,6-trimethyl-4-oxo-2-cyclohexene-1-carboxylate) which has a cyclohexene structure. The double bond in the 2,3 position of the C<sub>10</sub>-ester ketone was isomerised to the 1,2 position followed by the simultaneous protection of the ketone, in the form of a ketal. The ester function of the C<sub>10</sub>-ester acetal underwent reduction to afford an allylic alcohol on which an asymmetric Sharpless epoxidation was carried out using (-) diethyl tartrate affording (+)-(1R,2R)-1,2-Epoxy-4,4-ethylenedioxy-2,6,6-trimethylcyclohexane-1-methanol. The alcohol was oxidised by a Swern oxidation to the aldehyde in order to carry out a chain elongation. The side chain was prepared from 3,3-dimethylacrylic acid, which was first esterified then brominated to 4-bromosenecioate. The bromine was then replaced with triethyl phosphite, producing the C<sub>5</sub>-phosphonate. Chain elongation by Horner-Emmons reaction was carried out on the aldehyde with the phosphonate, which resulted in a mixture of *E* and *Z* C<sub>15</sub>-esters. Since the yield of the desired *Z* isomer, was very low, the *E* isomer was used to synthesize *trans*-xanthoxal, since this can be converted to the desired xanthoxal by uv radiation. The acetal was then removed followed by a simultaneous reduction of the ketone and ester groups to their corresponding alcohols using DIBAL. This reaction was only partially successful because only the keto group was reduced. The ester was then reduced with LiAlH<sub>4</sub> to form the primary alcohol, which was then selectively oxidised to the target molecule, the aldehyde (*trans*-xanthoxal).

The synthesis of xanthoxal described here highlights the difficulties encountered when introducing the requisite functional groups on the cyclohexene ring skeleton to afford *trans*-xanthoxal. The pitfalls encountered during the reaction sequence are discussed and solutions are presented. Although the planned synthetic sequence did not produce the correct stereoisomer, procedures are available to convert it to the desired isomer.

# 1. Introduction

## 1.1 History of Abscissic Acid

The research on plant growth inhibitors was initiated by several groups whose work was apparently not related initially but eventually led to the discovery of abscissic acid (ABA), which was later shown to be a plant hormone with important roles in plant growth and development and plant response to stress.

When the discovery of auxin suggested that low molecular weight compounds regulated plant growth, researchers attempted to explain all plant physiological phenomena in terms of changes in auxin levels.<sup>1</sup> However, it was simultaneously noticed by them that substances interfering with *Avena* coleoptile growth were present in this standard bioassay for auxin. Using this bioassay Hemberg found that potato peels contained growth inhibitors at high levels, which decreased under conditions that break dormancy.<sup>2</sup> He also showed the presence of a similar inhibitor in buds of *Fraxinus excelsior* and found a correlation between the levels of inhibitor and the degree of bud dormancy.<sup>3</sup> Thus, he pointed out the possibility that levels of both auxin and inhibitor regulated plant growth.

In 1952, Bennet-Clark *et al.*<sup>4</sup> used a new technique (paper chromatography coupled with a bioassay which they introduced) to measure the amount of auxin in plant tissue extracts. In addition to growth promotion by auxin, a constant feature was a zone of growth inhibition at a  $R_f$  of *ca.* 0.7 which was called “inhibitor  $\beta$ ”.<sup>5,6</sup> Subsequently this growth inhibitor was shown to be widely distributed in various plants species. Phillips and Wareing<sup>7</sup> investigated the changes of promoter and inhibitor levels in terminal buds and leaves of *Acer pseudoplatanus*. The levels of inhibitor in terminal buds was high in October, declined in winter, and reached a minimum in June. The changes in inhibitor levels in leaves was opposite to those in buds, suggesting that the inhibitor was formed in leaves in summer and moved to terminal buds at the beginning of autumn. These authors therefore suggested that the seasonal changes were related to photoperiodism and examined the inhibitor levels in *Acer* seedlings grown under long and short-day

conditions.<sup>8</sup> Growth tests showed that the inhibitor levels in the short day-treated seedlings were significantly higher than those in the long day-treated seedlings. Upon finding that inhibitor was also present in leaves of *Betula pubescens*, they applied crude inhibitor to leaves being grown under long day photoperiod. It was found that leaf growth was inhibited and that the terminal buds became dormant.<sup>9</sup> Wareing then called the inhibitor “dormin” after its ability to induce dormancy.

Wisterias bloom from the base of flower clusters to the apex, but only the first one or two flowers fructify while others abort. If all flowers fructified, competition for nutrition would occur and not all seeds could mature. Van Stevenick investigated the effect of fruits on the abscission of *Lupinus luteus* flowers, and found that abscission of upper flowers was accelerated by the presence of lower immature seeds. Upon removal of the lower immature seed, the flowers did not fall. This result suggested that an abscission-accelerator was formed in the immature seeds.<sup>10</sup> Rothwell and Wain<sup>11</sup> purified the abscission-accelerator found in fruit of pods *Lupinus luteus* to obtain a crystalline solid but did not succeed in determining its structure.

The successful isolation and elucidation of the structure came from work carried out by Addicott's group on premature abscission of cotton bolls. Addicott *et al.*<sup>12</sup> found that the bases of immature fruit contained a substance inhibiting the response of *Avena* coleoptiles to auxin, and thus confirmed that the substance had a growth inhibitory activity on *Avena* coleoptiles. Further work carried out by Ohkuma *et al.*<sup>13</sup> succeeded in the isolation of 9 mg of a highly active substance from immature cotton fruits, and called it “abscissin II”. In 1965 Ohkuma *et al.* published a proposed structure of abscissin II (1, Fig.1.1), elucidated from UV, IR, NMR and MS<sup>14</sup> spectral data which was confirmed by synthesis.<sup>15</sup> The absolute configuration at C-1 was determined to be *S* in 1973. Cornforth *et al.*<sup>16</sup> purified dormin from *Acer* leaves and found that dormin was the same as abscissin II. Further the flower abscission accelerator of *Lupinus* was identified as abscissin II and the major component of inhibitor  $\beta$  was considered to be abscissin II.<sup>17,18,19</sup> Thus, abscissin II or “dormin” was shown to be widely distributed in plants and considered to play an important role in various physiological phenomena.

At the sixth International Conference on Plant Growth Substances, it was agreed to unify the three names (*i.e.* dormin, abscissin II and inhibitor  $\beta$ ) into a single name *abscisic acid* (ABA)<sup>20,21</sup> which reflects its historical antecedents and emphasises its structure. A convenient numbering system was proposed in 1985 by Boyer *et al.*<sup>22</sup>

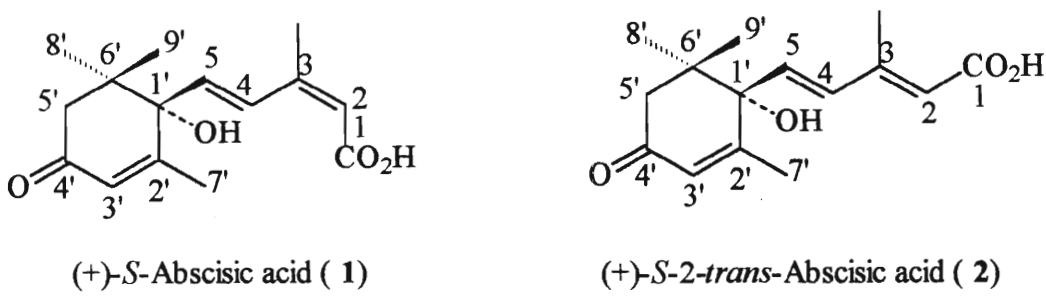


Figure 1.1

Like all other plant hormones, ABA has multiple physiological effects, influencing plant growth and development. It was first thought to be only associated with abscission and dormancy and considered strictly as an inhibitor. However, it is now known that ABA can also act as a promoter of, for example protein synthesis in seeds. ABA has a regulatory role in various plant processes (see **Table 1**) but is best known for its role in stomal closure and regulating water balance in plants. It also interacts with other plant hormones, regulating plant growth processes, and does not act merely as an inhibitor.

**Table 1** Plant Responses to ABA

Growth inhibition	Protein synthesis
Seed dormancy induction	Bud dormancy induction
Dormancy maintenance	Seed germination inhibition
Premature seed germination prevention	Embryo growth inhibition
Embryo growth promotion	Seed maturation

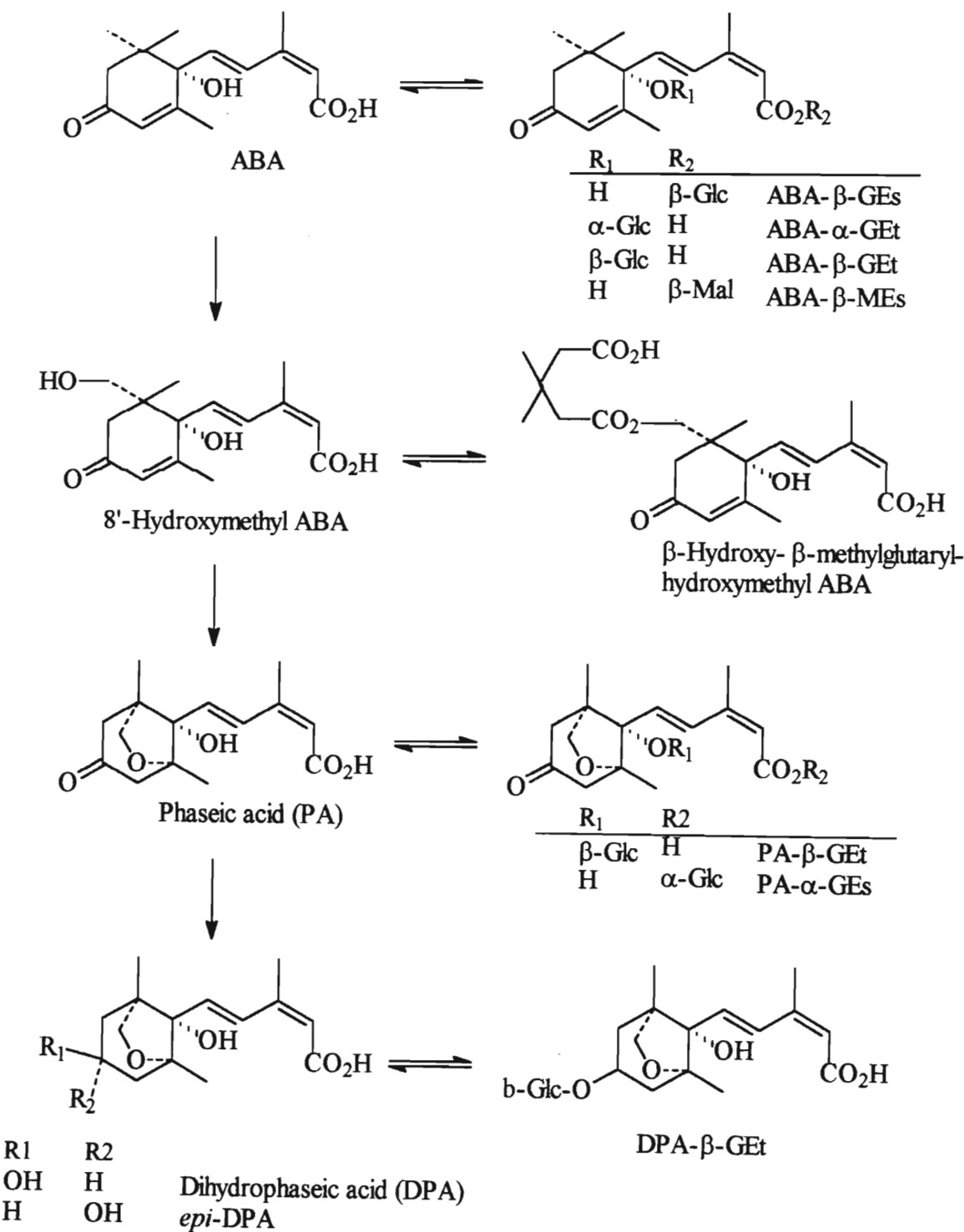
**Table 1. contd.**

Turgor pressure regulator	Ion transport regulation
Photosynthesis suppression	Stomal movement regulation
RNA synthesis modification	Photosynthate partitioning
Enzymic activity alteration	Gene expression modulation
Flowering promotion	Flowering inhibition
Fruit abscission promoter	Fruit ripening
Senescence promotion	Leaf abscission promotion
Counteraction of gibberellin-induced effects	

## **1.2 Biochemistry of Absciscic acid**

Absciscic acid applied to plant tissues is catabolised rapidly to more polar compounds by oxidation, hydroxylation, isomerisation, and conjugation with sugars or carboxylic acids. These compounds either possess very low or no biological activity, or serve as storage forms of ABA. The metabolites of ABA and the metabolic pathway have been elucidated by labelling studies and employment of HPLC and spectrometries on the micro scale. In 1986, Hirai<sup>23</sup> suggested an overall metabolic pathway of ABA metabolism (Fig.1.2). Absciscic acid isolated from plants is normally accompanied by a biologically inactive species, 2-*trans*-ABA (*t*-ABA) (2, Fig.1.1). It was initially thought to be formed from ABA during extraction because of photoisomerization. However, tissues grown in the dark and supplied with labelled ABA contained *t*-ABA.<sup>24,25</sup> The natural existence of *t*-ABA has been confirmed by many reporters and it is likely to be formed *in vivo* by an enzymatic reaction, rather than photoisomerisation. Since the first report on the presence of a  $\beta$ -glucosyl ester of ABA (ABA- $\beta$ -GEs) in immature seeds of yellow lupin several other workers have characterised conjugates of ABA by external applications of ABA to various plant tissues. Although the  $\beta$ -glucosyl ester of ABA appears to be widespread in plants, it has only been identified in a few instances.<sup>26,27,28</sup> Two compounds, 1'-*O*- $\alpha$ -glucoside of ABA (ABA- $\alpha$ -GEt) and 1'-*O*- $\beta$ -glucoside of ABA (ABA- $\beta$ -GEt) were identified as metabolites of ABA from extracts of tomato plants administered with ( $\pm$ )-[2-<sup>14</sup>C]-ABA.<sup>29</sup> During the course of studies on the role of ABA in dormancy,  $\beta$ -maltosyl ester of ABA (ABA- $\beta$ -MEs) was

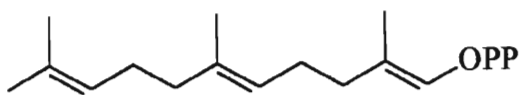
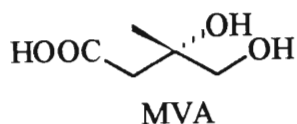
found in the bark and wood of birch trees (*Betula pubescens*).<sup>30</sup> These conjugates are considered to be storage forms of ABA since they can release free ABA reversibly. The metabolite 8'-hydroxymethyl ABA (HMABA) isolated from tomato shoots supplied with ABA<sup>23,31</sup> is derived only from the (+)-enantiomer of ABA<sup>23</sup> where the 8'-methyl group is hydroxylated.<sup>32</sup> The enzyme which catalyses this ABA-hydroxylation was isolated from cell-free extracts of *Echinocystis lobata* liquid endosperm.<sup>33</sup>



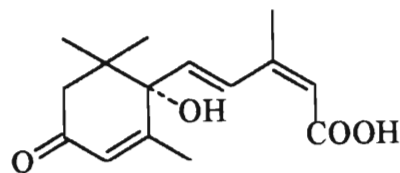
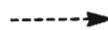
Recent studies have demonstrated that the 8'-ABA hydroxylase is an inducible cytochrome. A new conjugate of HMABA,  $\beta$ -hydroxy- $\beta$ -methylglutarylhydroxymethylABA (HMG-HOABA) was isolated from immature seeds of *Robinia pseudoacacia* by Hirai *et al.*<sup>34</sup> This conjugate, which seems to play an important role in the metabolism of ABA during seed maturation, is easily hydrolysed to HMG and phaseic acid (PA) in alkali. Since it has not been reported to be present in other plant species it may be specific to *Robinia pseudoacacia*. Phaseic acid is a natural metabolite, which has some biological activity. Although feeding experiments showed that  $(\pm)$ -[2-<sup>14</sup>C]-ABA and [2-<sup>14</sup>C]-PA were both metabolised to dihydrophaseic (DPA),<sup>35,36</sup> Sondheimer *et al.*<sup>37</sup> reported that  $(-)$ -[2-<sup>14</sup>C]-ABA was not metabolised to PA.

### **1.3 Absciscic Acid Biosynthesis**

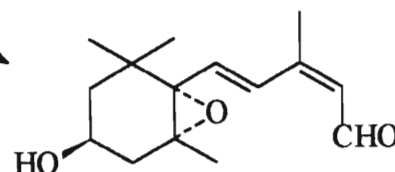
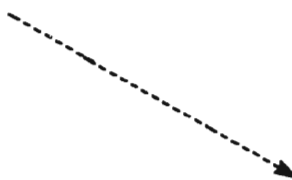
ABA, a C<sub>15</sub> compound, is a typical sesquiterpene, which consists of 3 isoprene units. It was initially postulated that ABA is synthesised from a (3*R*)-mevalonic acid (MVA). This is not necessarily true, given recent indications that plants contain a non-mevalonate pathway of isoprenoid synthesis. Several studies have demonstrated the synthesis of ABA from labelled MVA.<sup>22,38,38</sup> Only natural (+)-(3*R*)-MVA was incorporated into ABA by slices of avocado fruit while  $(-)$ -(3*S*)-MVA was not.<sup>39,40</sup> Since all terpenoids were thought to be derived from MVA by a well characterised pathway, it was expected that ABA should be similarly produced in higher plants. Although the stereochemical origin of the carbon and hydrogen atoms of ABA have been confirmed using stereochemically labelled MVA, the close resemblance of its structure to that of carotenoids led to the suggestion that ABA may be derived from carotenoid breakdown.<sup>41</sup> Taylor and Burden<sup>42</sup> demonstrated that photolytic cleavage of violaxanthin, a C<sub>40</sub> carotenoid, resulted in the production of a C<sub>15</sub> intermediate characterised as xanthoxal (a naturally occurring compound in a variety of plants) **Fig. 1.3**. While in 1972, Firm and Friend<sup>43</sup> showed that xanthoxal (Xan) could also be



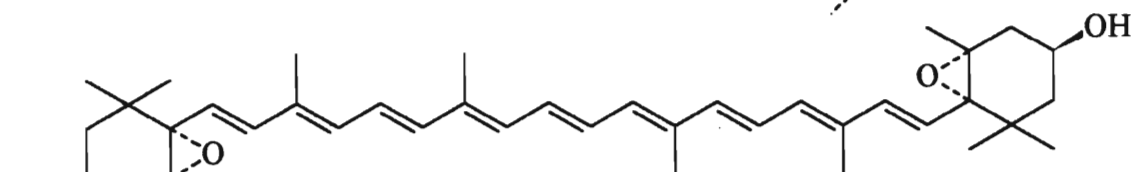
Farnesylpyrophosphate (C<sub>15</sub>)



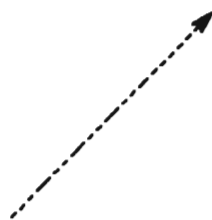
ABA



Xanthoxal



Violaxanthin (C<sub>40</sub>)



**Figure 1.3** Alternative Pathways for the biosynthesis of ABA

produced by the enzymatic cleavage of violaxanthin with lipoxygenase. In addition, Burden and Taylor<sup>44</sup> demonstrated the conversion of xanthoxin to ABA in shoots of bean (*Phaseolus vulgaris*) and tomato (*Lycopersicon esculentum*) when fed in radioactive form. These results supported the hypothesis that ABA was derived from

MVA via the carotenoid biosynthetic pathway. Evidence for a direct pathway operating in higher plants was scant. Robinson<sup>45</sup> fed <sup>14</sup>C-labelled phytoene, the first C<sub>40</sub> compound in the carotenoid biosynthetic pathway, together with <sup>3</sup>H-labelled MVA, the precursor of all terpenoids, to avocado fruit. Both <sup>14</sup>C and <sup>3</sup>H were found in isolated β-carotene, while the ABA recovered was labelled only with <sup>3</sup>H. This was taken as evidence that MVA was converted directly into ABA and not via a carotenoid. An alternative explanation was given by Grumbach<sup>46</sup> who used similar dual labelling studies. He obtained evidence that two pools of β-carotene existed, one small that provided precursors for xanthophyll synthesis and a second much larger, possibly located in the photosystems. He suggested that two spatially separate biosynthetic pathways supplied these two pools. Since Robinson did not examine the presence of <sup>14</sup>C label from phytoene in the xanthophylls, this work was regarded as inconclusive. For over 30 years there has been a debate as to whether ABA synthesis occurs either via a direct route from MVA through a C<sub>15</sub> intermediate, such as farnesyl-diphosphate (FPP) or derived indirectly as a cleavage product (C<sub>15</sub> compound) from a C<sub>40</sub> carotenoid such as violaxanthin (Fig. 1.3). Due to the fact that ABA is present in very low concentrations in plants, and that presumed precursors were poorly incorporated into ABA when applied in radioactive form, progress into the elucidation of ABA biosynthetic pathway was slowed. The inability to unequivocally characterise ABA intermediates, coupled with poor conversion of presumptive precursors to ABA, brought work on biosynthesis in plants to a virtual halt by the mid 1970s. In 1977, the discovery that a rose pathogen, the fungus *Cercospora risicola*, produces and excretes large quantities of the naturally occurring enantiomer of ABA into its growth medium initiated work into on the biosynthetic pathway in that organism.<sup>47</sup> Hoping that the pathway in *Cercospora risicola* was similar or identical to the pathway in higher plants, it was thought that the large production of ABA by the fungus would allow the pathway to be determined more readily. The discovery also led to the investigation of ABA production in other fungi. While studies with *Cercospora risicola* continued, investigators were restimulated to look at ABA biosynthesis in plants using different techniques to those used in earlier studies. Although the relative importance of each pathway remains unknown, results of these studies are in support of the indirect pathway.<sup>48,49,50</sup>

### 1.3.1 Direct Biosynthetic Pathway

#### 1.3.1.1 ABA biosynthesis in plants

Although radioactive ABA can be recovered from plant tissues to which radioactive MVA has been applied, no radioactive intermediate were identified. The intermediates might be unstable and easily converted to ABA. In a structure-activity relationship study where many ABA-derivatives were synthesised to compare their biological activities with that of ABA, it was found that certain compounds were converted to ABA by plants and or plant tissues. Reduction of ABA with  $\text{NaBH}_4$  gives *cis*- and *trans* ABA-diol (3) and (4), which were automatically oxidised to ABA in aqueous solution.<sup>51</sup> Since such unstable compounds are most probably candidates for the precursors of ABA a cold trap technique was employed to determine this.  $[2\text{-}^{14}\text{C}]$ -MVA was applied to avocado fruit, and after addition of unlabelled ABA-diol as a cold trap it was recovered. No radioactivity was incorporated into the recovered ABA-diol indicating that neither the *cis*- nor *trans*-ABA-diol was involved in the biosynthesis of ABA.

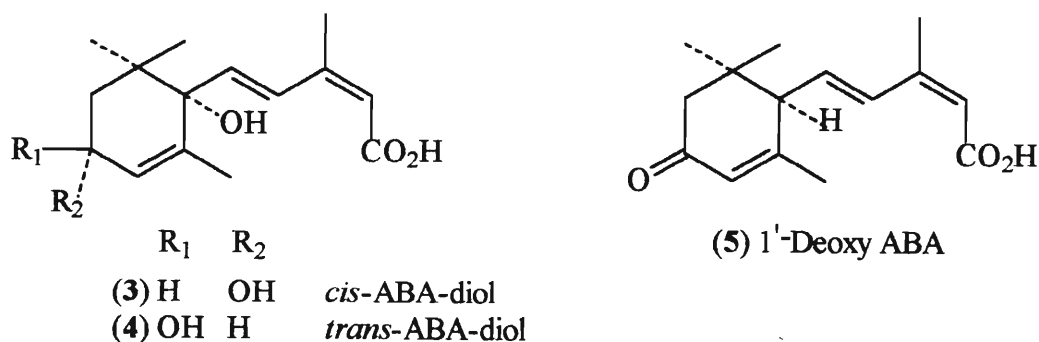


Figure 1.4

1'-Deoxy ABA (5) was synthesised to estimate the contribution of the 1'-hydroxyl group to the biological activity. The similar activity of 1'-deoxy ABA to ABA was interpreted as indicative of conversion of 1'-deoxy ABA to ABA by enzymatic hydroxylation in plants. However, a feeding experiment employing  $\alpha$ -ionylideneacetic

acid showed that 1'-deoxy ABA was not metabolised to ABA in plants. Although Mallaby<sup>52</sup> showed that 1'-deoxy ABA is hydroxylated at C-1' in air to give ABA, the hydroxylation does not seem to occur in plants, and evidence that 1'-deoxy ABA is a precursor of ABA has not been confirmed.

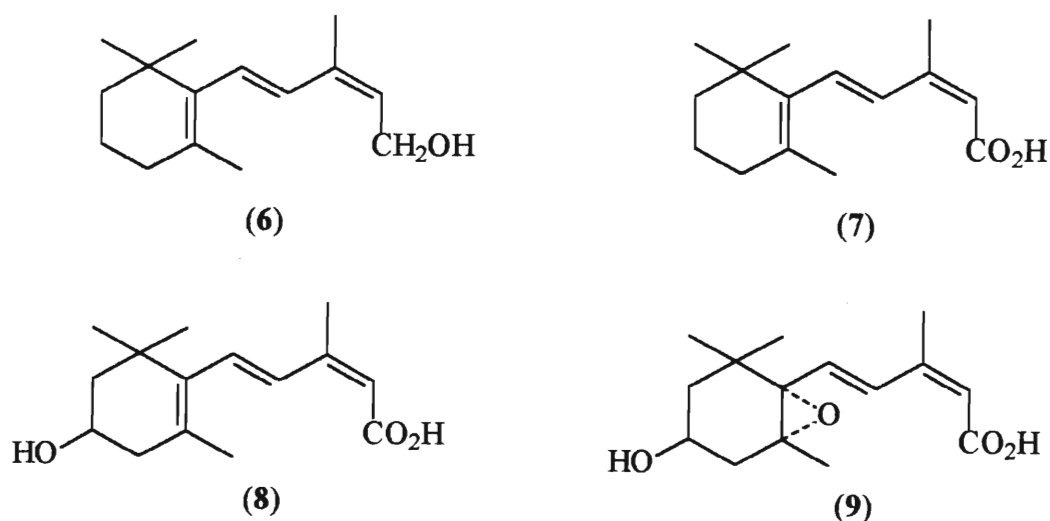
### 1.3.1.2 ABA Biosynthesis in Fungi

The natural occurrence of 1'-deoxy ABA in fungi was found and fungi seemed to metabolise this compound to ABA. The biosynthesis of ABA in fungi has been intensively investigated since Assante and co-workers<sup>53</sup> reported that ABA is produced by *Cercospora rosicola*. Since fungi produce relatively large amounts of ABA, detection of labelled intermediates should be easier than in plants. Bennett *et al.*<sup>54</sup> confirmed that *C. rosicola* biosynthesised ABA via the normal isoprenoid pathway.

Mycelia of *C. rosicola* were cultured in a liquid medium containing [1,2-<sup>13</sup>C]-sodium acetate, and ABA labelled with <sup>13</sup>C was isolated by HPLC. This result showed that six intact acetate units and three carbons derived from acetate were incorporated into ABA. This pattern of labelling was in complete agreement with the biosynthesis of ABA via the isoprenoid pathway as in higher plants. Neill *et al.*<sup>55</sup> proposed that 1'-deoxy ABA is the immediate precursor of ABA in *C. rosicola*. [2-<sup>3</sup>H]-Mevalonolactone was applied to the fungus at the onset of the period of rapid ABA production. The chromatogram indicated that in addition to the peak of ABA at  $t_R$ 16 the peak at  $t_R$ 21.5 min possessed radioactivity. From spectral data, the compound corresponding to the latter peak was identified as 1'-deoxy ABA. Although it has been believed that 1'-deoxy ABA is automatically converted to ABA, it was found that 1'-deoxy ABA is a stable compound. [3-Me-<sup>3</sup>H]-1'-deoxy ABA fed to cultures of *C. rosicola* was converted into ABA in a good yield of 11%. This result strongly suggested that 1'-deoxy ABA is the immediate precursor in *C. rosicola*.

Since the biosynthetic pathway was supposed to involve successive oxidation of  $\alpha$ -ionylidene derivatives, a feeding experiment with [3-Me-<sup>2</sup>H]- $\alpha$ -ionylideneethanol was

carried out.<sup>56</sup>  $^2\text{H}$ -labelled  $\alpha$ -ionylidenethanol and  $\alpha$ -ionylideneacetic acid were applied to *C. rosicola* and, after methylation, the acid extracts were analysed by GC/MS. The result showed that both compounds were converted to 1'-deoxy ABA and ABA in high yield. By contrast, the 2-*trans* isomers of the  $\alpha$ -ionylidenethanol and  $\alpha$ -ionylideneacetic acid were converted to the 2-*trans* isomer of 1'-deoxy ABA but not incorporated into ABA. This suggested that isomerisation of the 2,3-double bond precedes, or is concomitant with, the formation of the first unsaturated cyclic intermediate. On the other hand, neither  $\beta$ -ionylidenethanol (6) nor  $\beta$ -ionylideneacetic acid (7) were incorporated into 1'-deoxy ABA.

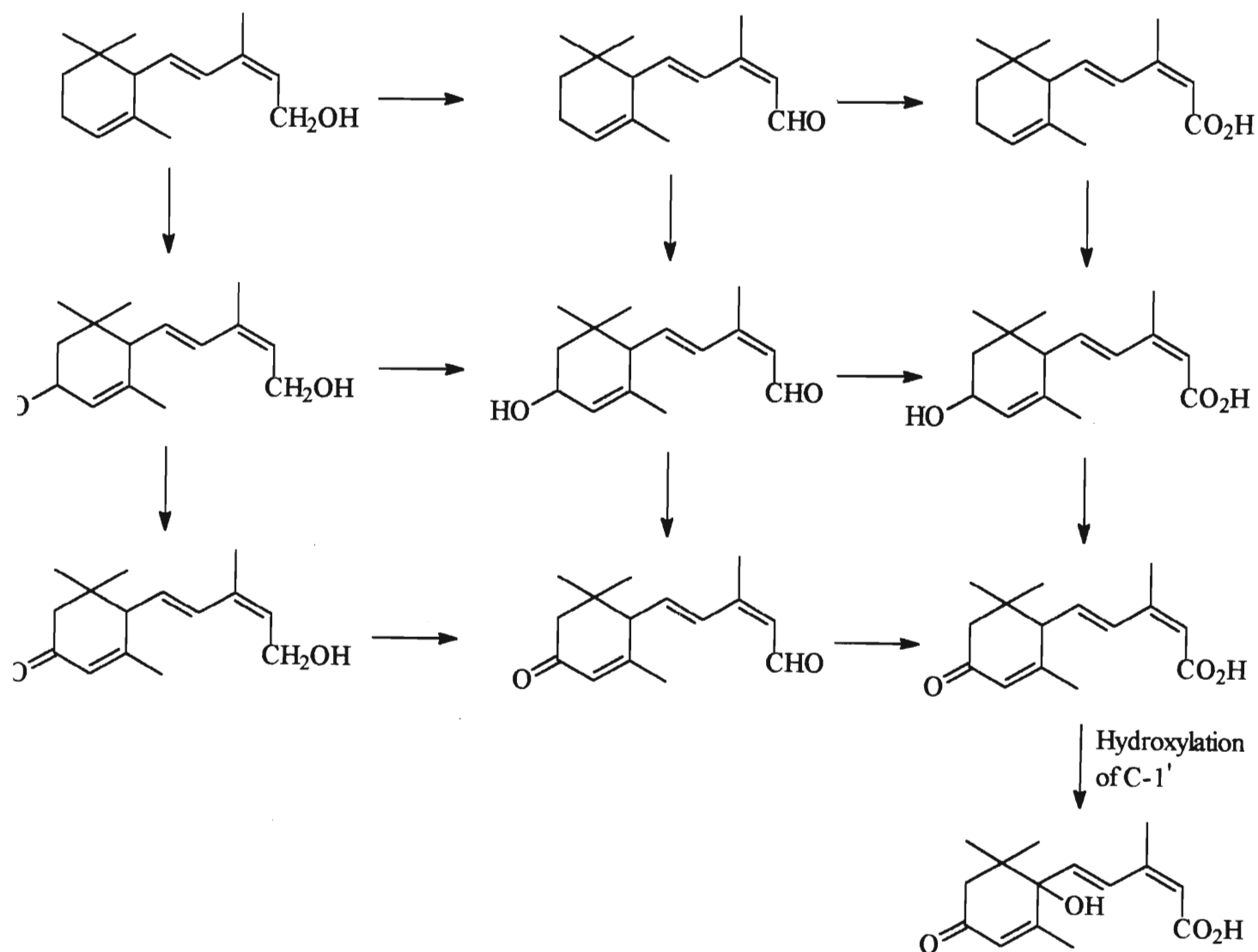


**Figure 1.5**

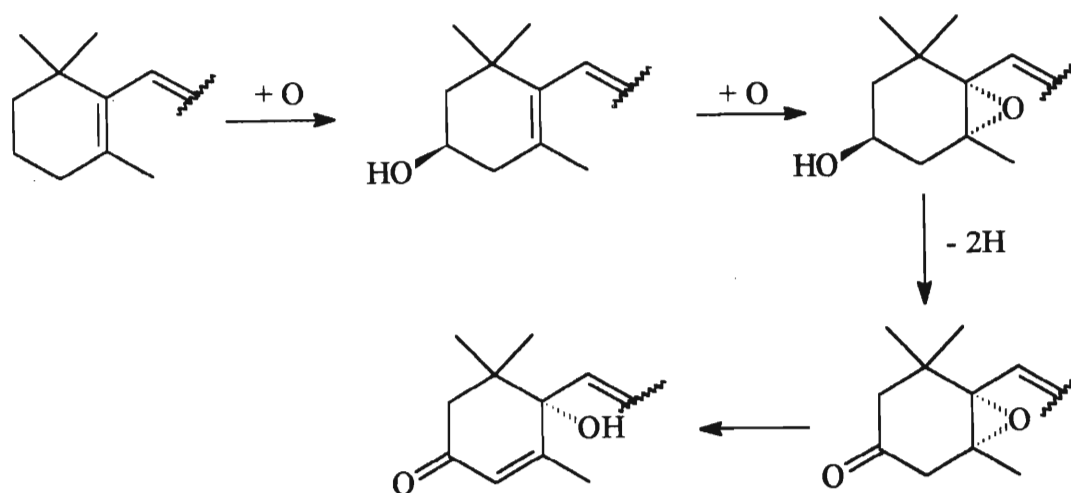
Based on these results, Neill *et al.*<sup>54</sup> proposed the biosynthetic pathway for ABA as shown in Fig 1.6. They carried out a similar experiment with *Vicia faba*, and suggested that this biosynthetic pathway is followed in higher plants.

Oritani and Yamashita<sup>57</sup> showed that 2-*cis*- $\beta$ -ionylideneacetic acid (7) was metabolised to ABA in tomato shoots and seedlings of rice and soybean. Since 4'-

hydroxy-2-*cis*- $\beta$ -ionylideneacetic acid (**8**) and (+)-(1'*S*,2'-*S*,4'*S*)-2-*cis*-xanthoxin acid (**9**) are rapidly converted into ABA, the conversion pathway of 2-*cis*- $\beta$ -ionylideneacetic acid to ABA has been proposed by Milborrow and Garmston<sup>58</sup> (Fig. 1.7).



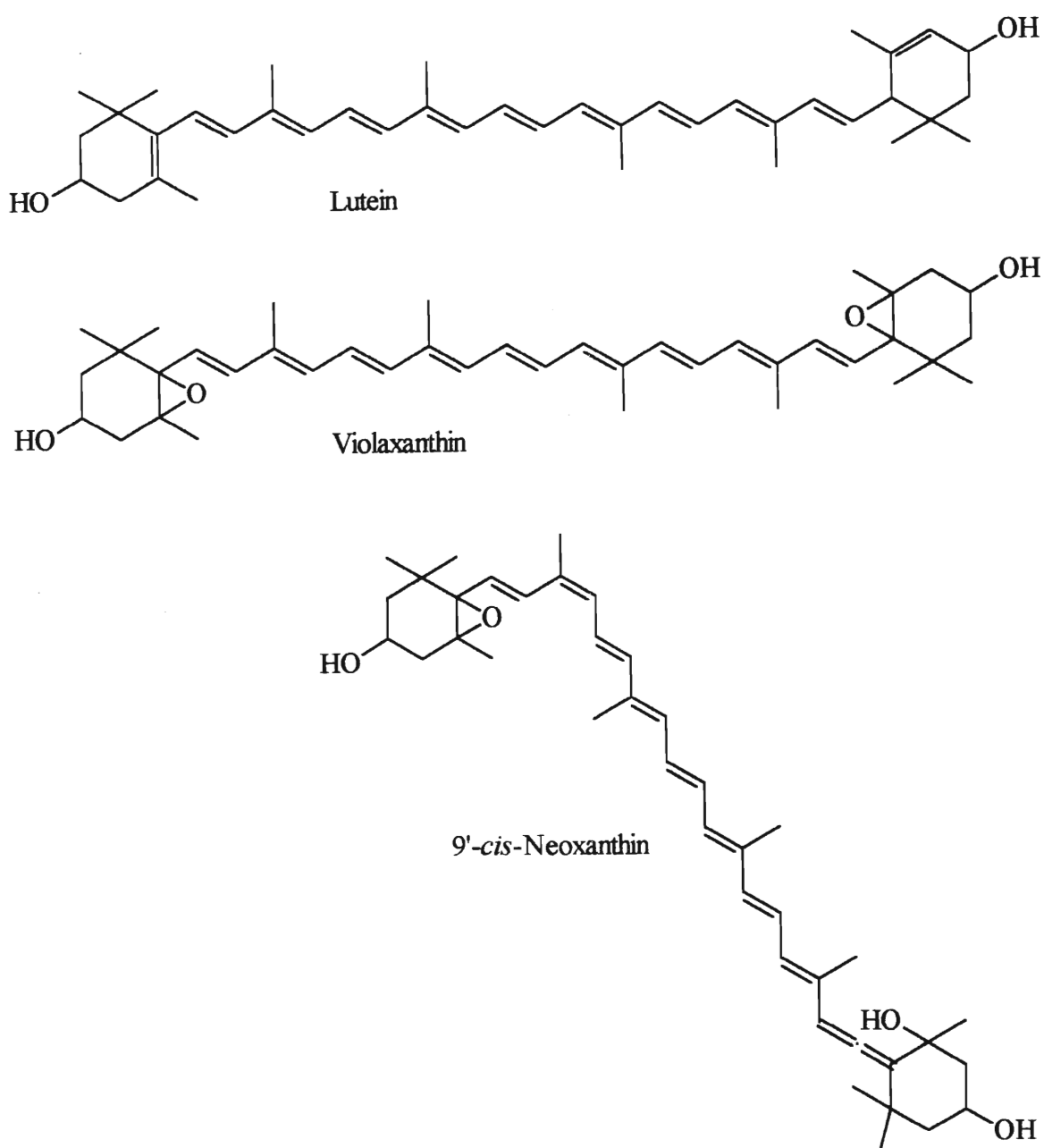
**Figure 1.6** Possible biosynthetic pathway in *C. rosicola*



**Figure 1.7** Oxidative pathway of Xanthoxal

### 1.3.2 Indirect Biosynthetic Pathway

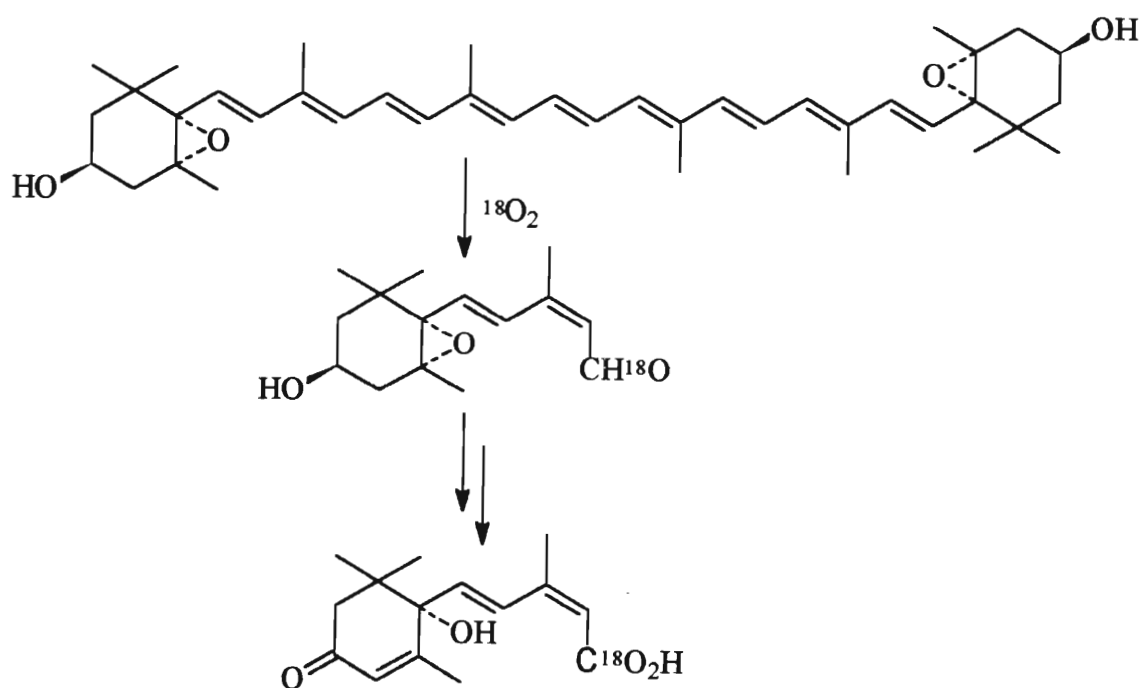
The problem in studying the ABA biosynthetic pathway in higher plants was the poor incorporation of radioactive precursors such as labelled MVA and  $\text{CO}_2$  into ABA. This led Milborrow to propose that the chloroplast is the site of ABA synthesis on the assumption that transport of  $^{14}\text{C}$ -MVA across the chloroplastic membrane was the rate-limiting step in incorporation. He reported that lysed chloroplasts incorporated  $^{14}\text{C}$ -MVA in ABA,<sup>59</sup> while other investigators reported that chloroplasts did not incorporate  $^{14}\text{C}$ -MVA into ABA.<sup>60</sup> An explanation for this result is that ABA is derived from preformed precursors present at high levels relative to ABA, and these precursors are synthesized at low rates in mature leaves. The consequence of preformed precursors with such attributes is that radioactive compounds will be incorporated into them very slowly and the radioactivity that is incorporated will be diluted by the high concentration of precursor. The major leaf xanthophylls, such as lutein, violaxanthin and neoxanthin, fit the requirements for such a precursor (**Fig.1.8**). They are present in levels more than  $10^5$  greater than ABA in unstressed leaves and their rates of synthesis in mature leaves are low.



**Figure 1.8** Structures of several leaf xanthophylls.

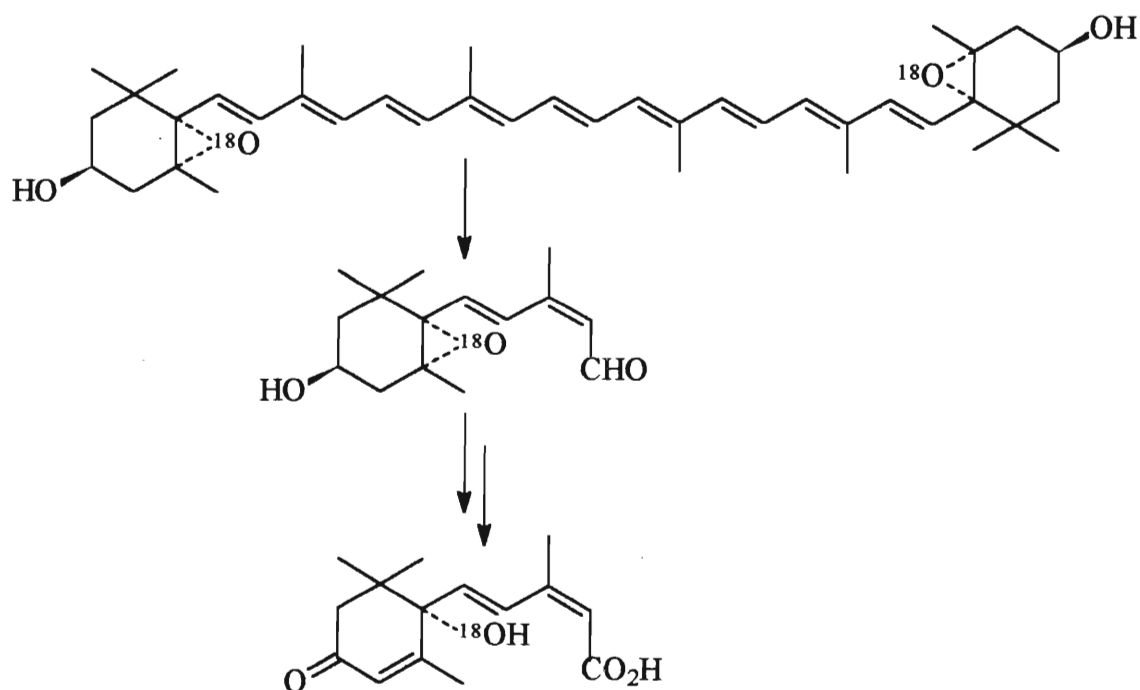
Overwhelming evidence in support of an indirect pathway was accumulated; for example several corn mutants which lacked the ability to synthesize carotenoids due to specific defects in their biosynthetic pathway were found to have a reduced ability to accumulate ABA in their leaves and roots.<sup>61</sup> In addition, inhibitors of carotenoid synthesis, such as fluridone and norflurazon also inhibited the accumulation of ABA under some conditions.<sup>62,63</sup>

A crucial experiment of a different type performed by Creelman and Zeevaart<sup>64</sup> also pointed to the possible role of xanthophylls as ABA precursors. They found that ABA extracted from stressed leaves of *Phaseolus vulgaris* and *Xanthium strumarium* incubated in the presence of  $^{18}\text{O}_2$  was labelled with only one  $^{18}\text{O}$ , located in the carboxyl group and not in the ring. Their rationale was that if 1'-deoxy ABA is the immediate precursor to ABA in plants, as is apparent in *C. rosicola*, then one  $^{18}\text{O}$  atom should be incorporated into the ABA ring at the 1' position. This assumes that the hydroxyl oxygen is derived from  $\text{O}_2$  as would normally be expected. This result suggested that a) 1'-deoxy ABA is not the immediate precursor of ABA, at least in water stressed leaves and b) ABA formed in water stressed leaves was derived from a preformed precursor containing the oxygen which would become the 1' and 4' oxygens of ABA. One explanation for these results is that a xanthophyll, such as violaxanthin, was cleaved in the presence of oxygen to form an aldehyde containing  $^{18}\text{O}$ . The aldehyde in the case of violaxanthin cleavage would be xanthoxal. If xanthoxal was oxidised by hydrogenases when converted to ABA, there would only be one atom of  $^{18}\text{O}$  in the carboxyl position and the second oxygen atom would have been obtained from water which would not contain  $^{18}\text{O}$  (Fig. 1.9).



**Figure 1.9** Hypothetical cleavage of violaxanthin to xanthoxin by  $^{18}\text{O}_2$  with subsequent conversion to ABA.

Although these experiments suggested that xanthophylls are ABA precursors, no information was given about the identity of the xanthophylls. In order to test whether violaxanthin is an ABA precursor, intact leaves were treated so that the epoxide oxygens were partially replaced by  $^{18}\text{O}$ .<sup>65</sup> The leaves were then water stressed and both violaxanthin and ABA were isolated and analysed. If the ABA produced during the water stress period had been derived from violaxanthin containing  $^{18}\text{O}$  in its epoxide group, one would expect to observe  $^{18}\text{O}$  at the ABA 1'-hydroxyl group (Fig. 1.10). The results suggested that a portion of the ABA was derived from the violaxanthin that had been labelled with  $^{18}\text{O}$ , but this violaxanthin only accounted for 25% of the ABA produced. The participation of 9'-*cis*-neoxanthin also helps to explain why there was only an apparent 25% incorporation of  $^{18}\text{O}$  from the all-*trans*-violaxanthin into ABA.<sup>63</sup> A portion of the ABA would have been formed directly from the pool of unlabelled 9'-*cis*-neoxanthin thus diluting the incorporation of  $^{18}\text{O}$ . These results were confirmed and extended to tomato shoots by Parry *et al.*<sup>66</sup> Further evidence has been obtained from work with *Arabidopsis* ABA-deficient mutants which have a greatly reduced capacity to epoxidise zeaxanthin to violaxanthin and consequently to produce 9'-*cis*-neoxanthin.<sup>67</sup>



**Figure 1.10** Conversion of violaxanthin containing  $^{18}\text{O}$  in the epoxide to ABA containing  $^{18}\text{O}$  at 1'-hydroxyl position.

### 1.3.2.1 Xanthophyll ABA Precursors

The similarity between ABA and the end groups of certain xanthophylls was first noted by Taylor and Smith.<sup>40</sup> Photo oxidation of these xanthophylls possessing 5,6-epoxy and 3-hydroxy groups, such as violaxanthin and neoxanthin, gave rise to a neutral compound later identified as Xan, which inhibited cress seed germination<sup>41,68</sup> Xanthoxal was present in a variety of plant extracts together with its geometric isomer 2-*trans*-xanthoxal (*t*-Xan).<sup>69,70</sup> Xan is active in a range of bioassays while *t*-Xan possesses little or no biological activity.<sup>44</sup> Taylor and Burden<sup>71</sup> (1972) extracted xanthophylls from various plants materials and cleaved them using mild chemical oxidation. Under these chemical conditions it appeared that the ratio of *t*-Xan:Xan was determined by the ratio of the all-*trans* to 9-*cis* xanthophyll isomers extracted. Feeding experiments utilising <sup>13</sup>C and <sup>14</sup>C-labelled Xan and *t*-Xan have shown that *t*-Xan cannot act as a precursor to Xan or ABA.<sup>43,72</sup> Similar results have been obtained using cell-free systems capable of synthesising ABA from Xan.<sup>73</sup> From this it seemed likely, therefore that Xan and *t*-Xan have independent origins and that *in vivo* isomerisation of *t*-Xan to Xan does not occur. The levels of Xan in plant extracts are always much less than those of *t*-Xan, probably due to the facile conversion of Xan to ABA.<sup>74</sup>

Thus, any endogenous Xan precursor would have to possess a *cis* configuration corresponding to the 2,3-double bond of Xan (e.g. 9,10-double bond of carotenoids). In photosynthetic tissues the majority of plant carotenoids occur predominantly in the thermodynamically more stable all-*trans* configuration<sup>75,76</sup> although there are some exceptions. Leaf violaxanthin, for example, is 95-99% all-*trans*, but 9-*cis*-violaxanthin has been identified as a minor component<sup>77,78</sup> The 9,9'-di-*cis*-violaxanthin is a likely candidate for an ABA precursor, however, no trace of this or any other di-*cis* isomer has been detected in any leaf or root extract examined thus far.<sup>67,68</sup> In contrast, neoxanthin occurs predominantly as the 9'-*cis* isomer, with the all-*trans*-neoxanthin making up only 2-5% of the total.<sup>65</sup> While the relative composition of leaf xanthophylls is similar in higher plants,<sup>65</sup> the distribution in etiolated leaves and roots is much more varied.<sup>79,80</sup> In extracts of light-grown and etiolated leaves and roots of a variety of

species, 9-*cis*-violaxanthin and 9'-*cis*-neoxanthin have been identified as the potential Xan precursors. Although the levels of 9-*cis*-violaxanthin in both light-grown and etiolated leaves are similar to those of ABA, they are unaffected by stress, while levels of ABA increase 3- to 40-fold.<sup>67,68</sup> If 9-*cis*-violaxanthin was an ABA precursor, either being cleaved directly to generate Xan or *via* the conversion to 9'-*cis*-neoxanthin, then it must have a rapid turn over rate (up to 20-25 times an hour) to support the observed increases in ABA levels. The possibility of 9-*cis*-violaxanthin occurring as a result of isomerisation from the more abundant all-*trans* isomer cannot be discounted.

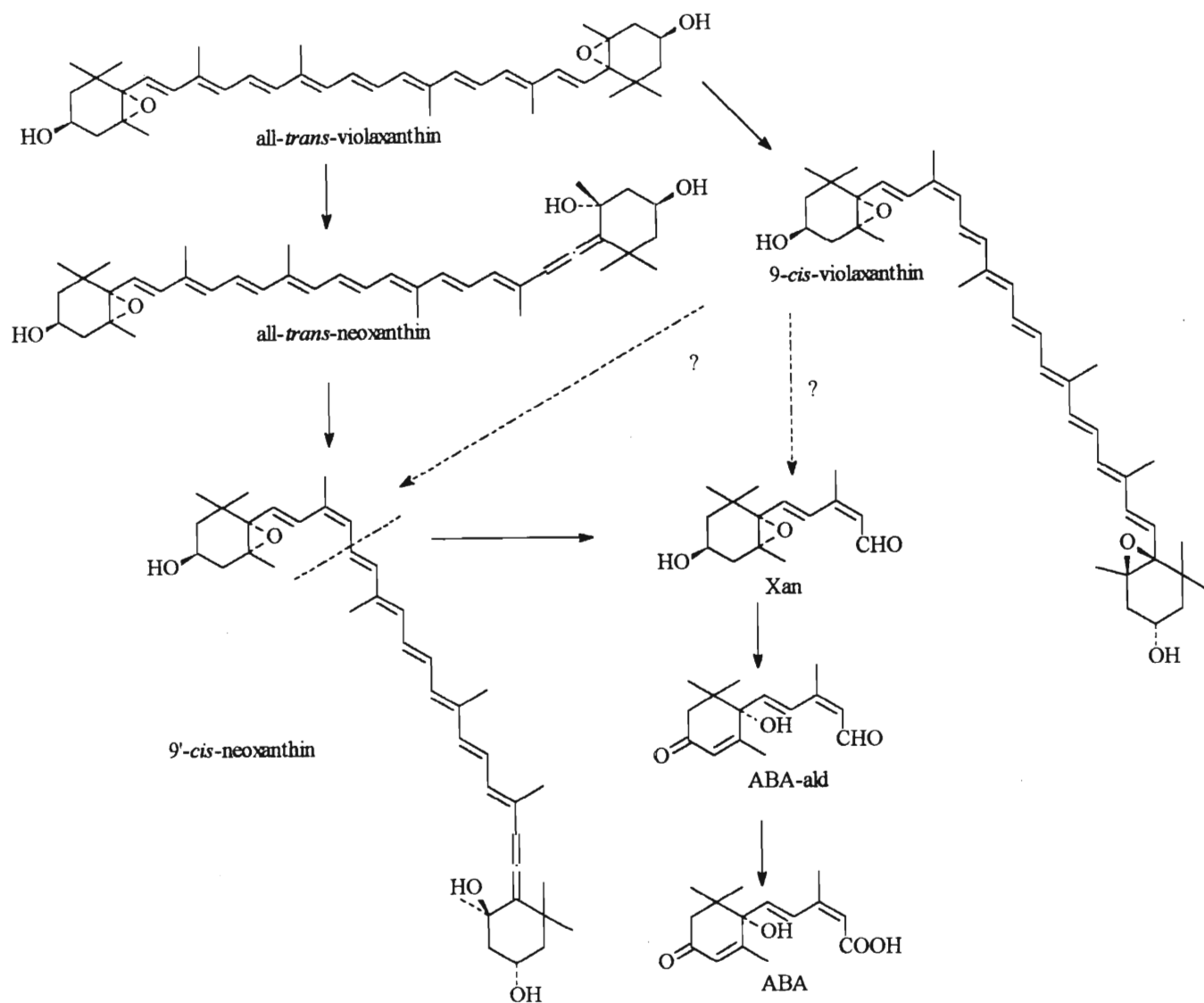
### 1.3.2.2 The Conversion of Violaxanthin to Neoxanthin

The stoichiometric decreases in xanthophyll levels, which accompany ABA synthesis in water-stressed etiolated *Phaseolus* leaves, are due to decreases in 9'-*cis*-neoxanthin (20-30%) and 9-*cis*-violaxanthin (65-80%). The relationship between these xanthophylls were investigated by Parry and Horgan.<sup>69</sup> Detached non-stressed leaves, and those pre-treated with cycloheximide, neither synthesize ABA nor show altered carotenoid levels.<sup>67</sup> All-*trans*-violaxanthin levels fell in stressed etiolated leaves but this isomer cannot generate Xan directly, and so must be converted into a 9-*cis* isomer prior to cleavage. Transfer of etiolated seedlings to light results in a general stimulation of carotenoid synthesis although the rates of accumulation for individual carotenoids vary widely. Fluridone, which inhibits carotogenesis at the stage of photoene dehydrogenase, was used to prevent the synthesis of new carotenoids after the transfer of etiolated seedlings to light. After 6-25 hrs, decreases occurred in the levels of all-*trans*-violaxanthin concomitant with increases in the levels of 9'-*cis*-neoxanthin. The combined levels of these two xanthophylls remained virtually constant, while the ratio of all-*trans*-violaxanthin:9'-*cis*-neoxanthin changed from *ca* 5:1 to 1:1.

These results suggest that all-*trans*-violaxanthin acts as a precursor of 9'-*cis*-neoxanthin, at least during the conversion of chloroplasts. This is consistent with the previous hypothesis regarding neoxanthin formation.<sup>65</sup> There was no significant change in the level of all-*trans*-neoxanthin or 9'-*cis*-violaxanthin during the conversion of all-*trans*-violaxanthin to 9'-*cis*-neoxanthin, making it impossible to predict which, if either, was an intermediate in the conversion.

To further investigate the relationship between violaxanthin, neoxanthin and ABA, extracts were made of etiolated *Phaseolus* seedlings grown on 50% D<sub>2</sub>O/H<sub>2</sub>O.<sup>69</sup> The total extent of deuteration, calculated from mass spectra, of ABA and phaseic acid was entirely consistent with the formation of ABA by 11'-cleavage of neoxanthin. Further experiments indicated that upon transfer of etiolated seedlings to light, violaxanthin and lutein were synthesised from newly-formed precursors, while neoxanthin was derived from an existing compound, most likely to be violaxanthin. It was first proposed by Li and Walton<sup>67</sup> (1990), and further supported by the findings of Parry *et al.*,<sup>68</sup> that the biosynthesis of ABA involves the pathway shown in Fig. 1.11. The rate-limiting step is believed to be the cleavage of 9'-*cis*-neoxanthin. The enzyme responsible for this cleavage is likely to have a short half-life as ABA levels in cycloheximide-treated *Phaseolus* leaves fell to half their normal levels within 4-5 hours.<sup>81</sup>

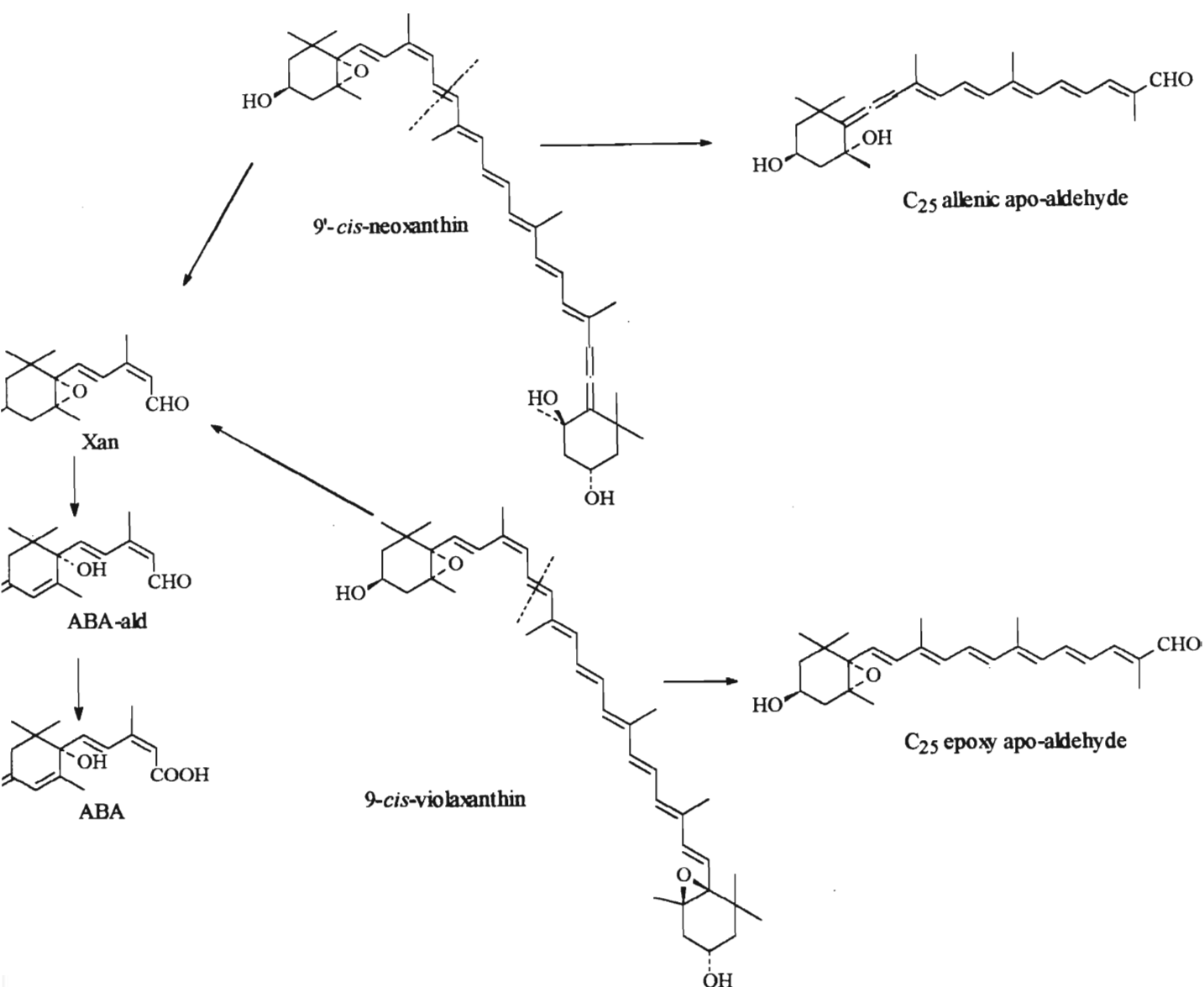
Employing an abscisic acid biosynthesising citrus cell-free system, Cowan and Richardson<sup>82</sup> investigated the metabolic interrelationship between carotenoids and abscisic acid. Biosynthetically prepared [<sup>14</sup>C]-all-*trans*-violaxanthin, [<sup>14</sup>C]-all-*trans*-neoxanthin and [<sup>14</sup>C]-9'-*cis*-neoxanthin were used as substrates and their *in vitro* conversion to ABA and related metabolites monitored. Results showed that ABA was more readily produced from 9'-*cis*-neoxanthin and supported the following precursor/product relationship: all-*trans*-violaxanthin → all-*trans*-neoxanthin → 9'-*cis*-neoxanthin → ABA.



**Figure 1.11** Pathway of ABA biosynthesis, showing the main (only?) route from *all-trans-violaxanthin*, via *all-trans-neoxanthin* to *9'-cis-neoxanthin*.

### 1.3.2.3 The Cleavage of *9'-cis-Neoxanthin*

The stoichiometry observed by Li and Walton<sup>67</sup> and Parry *et al.*<sup>64,70</sup> implies that one mole of xanthophyll is converted to one mole of ABA, and indicates that the cleavage reaction is a specific one, where *9'-cis-neoxanthin* is cleaved across the 11',12'-double bond to generate Xan. If this is so, then in addition to Xan, a  $C_{25}$  allenic apo-aldehyde or  $C_{25}$  epoxy apo-aldehyde should also be produced (Fig. 1.12)



**Figure 1.12** Specific cleavage of 9'-cis-neoxanthin and 9-cis-violaxanthin.

In order to investigate the nature of the *in vivo* cleavage of the xanthophylls, a range of allenic and epoxy apo-aldehydes ranging from C<sub>15</sub> to C<sub>27</sub> were synthesised by Parry and Horgan.<sup>69</sup> Extracts from a variety of organs [roots, etiolated and green leaves (stressed and non-stressed)] of *Lycopersicon* and *Phaseolus* were analysed, but no traces of any of the apo-aldehydes were detected.

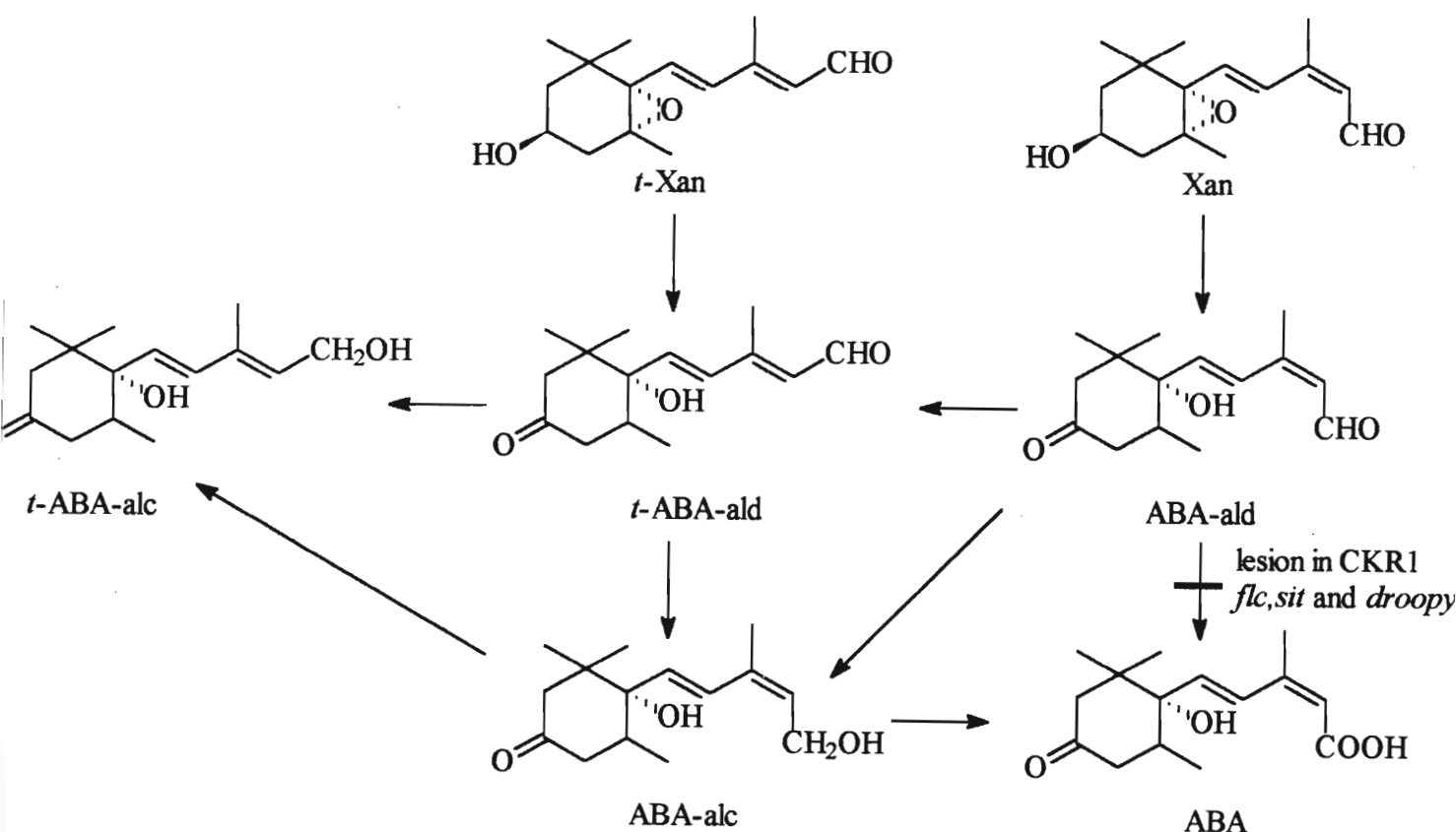
Numerous possible apo-carotenoids have been identified in extracts of plant and animal tissue but knowledge of the biochemistry of carotenoid catabolism is extremely

limited.<sup>83</sup> The majority of these apo-carotenoids are volatile (C<sub>9</sub>-C<sub>13</sub>) compounds but others range up to C<sub>30</sub>. The C<sub>25</sub> and C<sub>27</sub> epoxy apo-aldehydes of interest and their related alcohols, have been isolated from various fruits.<sup>84,85</sup> No compounds that could have been C<sub>25</sub> or C<sub>27</sub> apo-alcohols, apo-acids or esters of apo-acids were found to accumulate in stressed etiolated *Phaseolus* leaves.<sup>69</sup> Parry and Horgan<sup>69</sup> (1991) used lipoxygenase (LOX) to bleach solutions of neoxanthin and violaxanthin. Incubations interrupted with an anti-oxidant *tert*-butylated hydroxyquinoline contained low levels of C<sub>25</sub> or C<sub>27</sub> apo-aldehydes. Xan, *t*-Xan and the C<sub>15</sub> allenic apo-aldehyde were also produced but in low yields. The LOX/fatty acid free radical cleavage of xanthophylls appears to show no substrate specificity and results in complete fragmentation, producing molecules  $\leq$  C<sub>13</sub>. Since 9'-*cis*-neoxanthin is cleaved across the 11',12' double bond to generate Xan, it may be that a specific enzyme performs the first cleavage after which LOX and related enzymes rapidly degrade any C<sub>25</sub> apo-aldehydes released. A model system for the cleavage of 9'-*cis*-neoxanthin should be the formation of retinal from  $\beta$ -carotene. Surprisingly, although the product/precursor relationship of  $\beta$ -carotene and retinal was discovered over 70 years ago<sup>86</sup> there is little known about this reaction other than that it makes use of molecular oxygen.

#### 1.3.2.4 The Conversion of Xanthoxal to ABA

If 9'-*cis*-neoxanthin is the immediate pre-cleavage precursor of ABA, as it now seems likely,<sup>87</sup> then Xan must be the *in vivo* C<sub>15</sub> post-cleavage intermediate. Sindhu and Walton<sup>88</sup> made cell free systems from leaves of *Phaseolus vulgaris*, *Vigna radiata*, *Zea mays*, *Cucurbita maxima* and *Pisum sativum* and roots of *Phaseolus vulgaris* which had the ability to convert Xan to ABA. The 'Xan oxidising' activity was associated exclusively with cytosolic fractions. Experiments with leaves and cell-free systems of the wild-type and three ABA deficient mutants of *Lycopersicon* leaves showed that two of them, *Flacca* (*flc*) and *Sitiens* (*sit*), were unable to convert Xan to ABA.<sup>89</sup> 'Xan oxidising' activity was obtained from *Lycopersicon* leaves by including dithiothreitol

(DTT) in the extraction buffer. Combining cell-free extracts of *flc* and *sit* did not overcome their inability to convert Xan to ABA. The actual step of the pathway affected, and the immediate precursor to ABA, was identified by feeding a range of related compounds to these cell-free systems. Extracts of *flc* and *sit* were unable to convert ABA-aldehyde to ABA, showing the last step of the pathway does not involve a simple oxidation of the side-chain aldehyde to the carboxylic acid (**Fig. 1.13**).



**Figure 1.13** The latter C<sub>15</sub>, part of the ABA biosynthetic pathway. In normal plants Xan is converted via ABA-ald to ABA, whereas mutants such as *flc* accumulate *t*-ABA-alc.

Sindhu *et al.*<sup>90</sup> have fractionated the 'Xan oxidising' activity from *Phaseolus* leaves by differential precipitation with acetone. The two activities have been termed xanthoxin oxidase (Xan oxidase), which catalyses the two step reaction of Xan to ABA-aldehyde, and abscisic aldehyde oxidase (ABA-ald oxidase) which converts ABA-aldehyde to ABA. Extracts of *flc* and *sit* leaves were, as expected, deficient in ABA-ald oxidase

and not Xan oxidase.<sup>87</sup> When ABA-ald oxidase extracted from *Phaseolus* leaves was added to cell-free systems of *flc* and *sit*, conversion of Xan to ABA was observed. Walker-Simmons *et al.*<sup>91</sup> identified an ABA-deficient mutant of *Hordeum vulgare* (Az34) whose mutation is located in a gene controlling a molybdenum co-factor. The mutant was found to lack ABA-ald oxidase activity with a number of substrates including ABA-ald. Sindhu *et al.*<sup>87</sup> failed to detect ABA-ald oxidase from either turgid or stressed leaves of Az34, indicating that ABA-ald oxidase may be a molybdoenzyme.

In leaves of *flc* and *sit* a compound identified as 2-*trans*-ABA-alcohol (*trans*-ABA-alc) was found to accumulate, especially after water stress, and was proposed to be an ABA precursor.<sup>92</sup> Later feeding studies with [<sup>2</sup>H]ABA-ald revealed that while wild type leaves converted ABA-ald to ABA those of *flc*, *sit* and the *droopy* potato mutant reduced and isomerised ABA-ald to *trans*-ABA-alc<sup>93,94</sup> (Fig 1.13). This was further evidence for ABA-ald being the immediate ABA precursor. Parry *et al.*<sup>95</sup> characterised a new ABA deficient mutant of *Nicotiana plumbaginifolia* (CKR1) as being similarly unable to convert ABA-ald to ABA. Wild-type *Lycopersicon* and *Nicotiana* leaves converted Xan to ABA and *trans*-Xan to *trans*-ABA-alc while leaves of *flc* and CKR1 converted both Xan and *trans*-Xan to *trans*-ABA-alc. Both ABA-alc and *trans*-ABA-ald were converted to *trans*-ABA-alc and so the order of reduction and isomerisation is unclear<sup>96</sup> (Fig. 1.13). Mutants such as *flc*, *sit* and CKR1 are all ABA deficient but not ABA lacking; an explanation for this may have been found by Rock and Zeevaart.<sup>97</sup> Using <sup>18</sup>O<sub>2</sub> they have obtained evidence that some ABA-ald is reduced to ABA-alc which is then reoxidised by a monooxygenase to ABA. This may act as a minor pathway in normal plants as well as in mutants blocked in the conversion of ABA-ald to ABA.

In their investigation of the metabolic interrelationship of carotenoids and abscisic acid, Cowan and Richardson<sup>81</sup> found no evidence for the formation of ABA-aldehyde. In addition, all attempts to isolate ABA-aldehyde from *Citrus* exocarp were unsuccessful. However, the results of their work showed for the first time that terpenyl

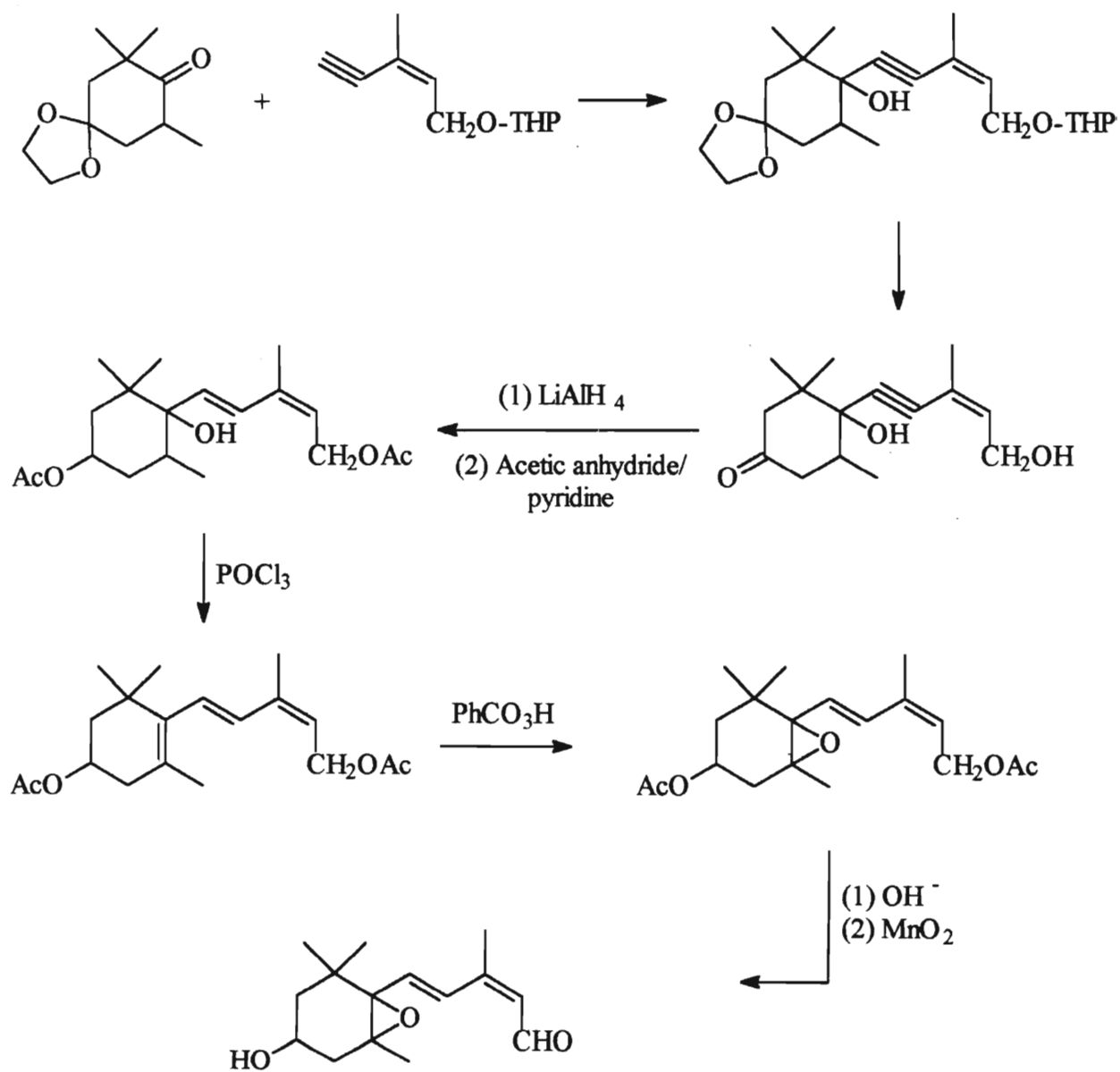
pyrophosphates,  $\beta$ -carotene, violaxanthin, neoxanthin and 9'-*cis*-neoxanthin, can be enzymatically converted to Xan and Xan-acid, which is then transformed to ABA *via* 1',4'-*trans*-ABAdiol. Previous work by Cowan and Richardson<sup>98</sup> is in support of the precursor role of 1',4'-*trans*-ABAdiol due to the fact that a cold pool trap of ABA caused accumulation of label from [2-<sup>14</sup>C]-MVA in 1',4'-*trans*-ABAdiol.

## **1.4 Syntheses of Xanthoxal**

To date there are 6 total syntheses of xanthoxal published in the literature.<sup>99,100,101,102,103,104</sup> However, only those syntheses which do not involve the painstaking separation of diastereomeric mixtures will be summarized here, as the attempted synthesis of xanthoxal described in this dissertation employs a stereoselective strategy. All the above syntheses describe the preparations of both xanthoxal (Xan) and *trans*-xanthoxal (*trans*-Xan). Of these, only 4 describe stereoselective methods<sup>98-101</sup> and in 3 cases Xan is obtained exclusively. All four methods are similar in principle with the only difference being the choice of starting material used. The rest of the chapter will be devoted to a review in which published syntheses of Xan are described, and which will serve as a means of comparing the synthetic route we employed with those of other workers.

Oritani and Yamashita reported the first total synthesis of Xan and *trans*-Xan (Scheme 1), in 1973<sup>105</sup> (cited in a paper by Burden and Taylor<sup>106</sup>). No details of the synthesis are given here, as we were unable to obtain the paper.

Scheme 1



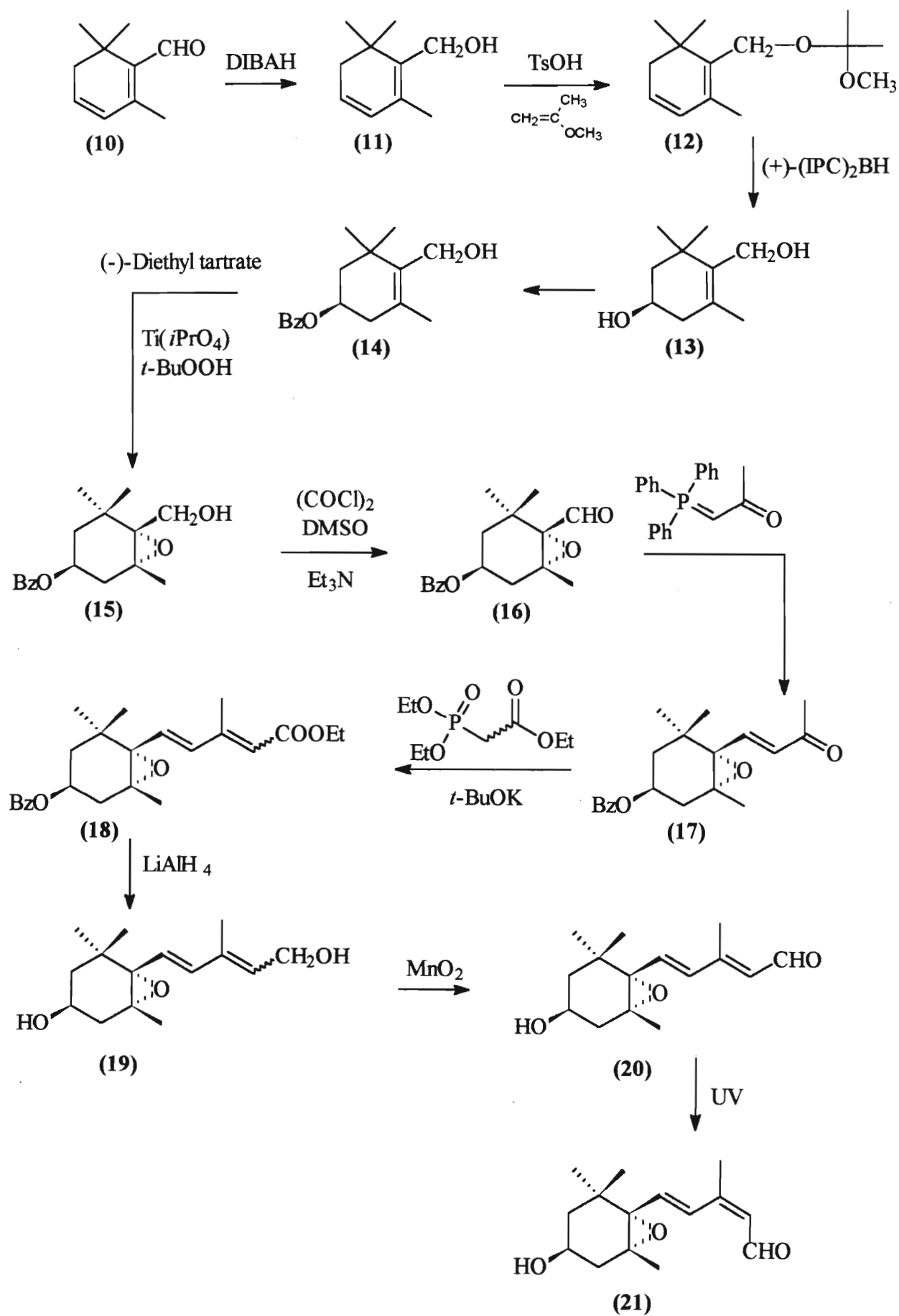
### 1.4.1 Synthesis from Safranal

Saffron, obtained by drying stigmas of *Crocus sativus* L., is a spice used for the flavouring and colouring of food. It contains a bitter principle, glycoside picrocrocin from which safranal (**10**) is obtained by hydrolysis.<sup>107</sup> This compound possesses the characteristic aroma of saffron.

After its isolation and identification in 1935,<sup>108</sup> several synthetic routes to safranal were published,<sup>109,110,111</sup> but none of these have much practical value since all reactions are complicated and give mixtures of aldehydes. Since neither safranal nor picrocrocin are commercially available and cannot be easily synthesized we did not view it as an ideal starting material.

A paper by Yamamoto and Oritani<sup>102</sup> describes the total synthesis of Xan and *trans*-Xan from the optically pure benzoate ester (**14**) of (*R*)-4-hydroxy- $\beta$ -cyclogeraniol (Scheme 2). This compound was easily obtained by the asymmetric hydroboration of safranol isopropenylmethylether (**12**) with (+)-(IPC)<sub>2</sub>BH.<sup>112</sup> The safranol isopropenylmethylether (**12**) was prepared from safranol (**11**), TsOH and isopropenyl methyl ether. safranol (**11**) was prepared from Safranal (**10**) using diisobutylaluminium hydride.<sup>112</sup>

Scheme 2

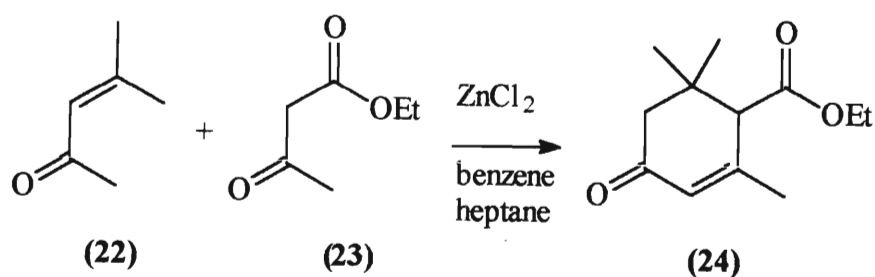


The alcohol (**14**) was stereospecifically converted to the (1*R*, 2*R*, 4*S*)-epoxide (**15**) via Sharpless asymmetric epoxidation with (-)-diethyl tartrate.<sup>113</sup> Swern oxidation of **15** gave aldehyde **16**. Stepwise elongation of the side chain was achieved by the Wittig reaction of **16** and subsequent Horner reaction of **17**. The ethyl ester (**18**) was converted to the allylic alcohol (**19**) by lithium aluminium hydride reduction and the following MnO<sub>2</sub> oxidation gave the (-)-(2*E*, 4*E*, 1'*S*, 2'*R*, 4'*S*)-xanthoxal (*trans*-Xan). The UV radiation of **20** gave an equilibrated mixture of **20** and its (2*Z*, 4*E*) isomer **21**, Xan. Facile separation of **20** and **21** was performed by preparative HPLC where the recovered **20** was repeatedly converted to **21**. Although this synthesis appears to be easy and high yielding it was not followed as the starting material *viz.*, safranal was difficult to obtain.

#### 1.4.2 Synthesis from Mesityl Oxide and Ethyl Acetoacetate

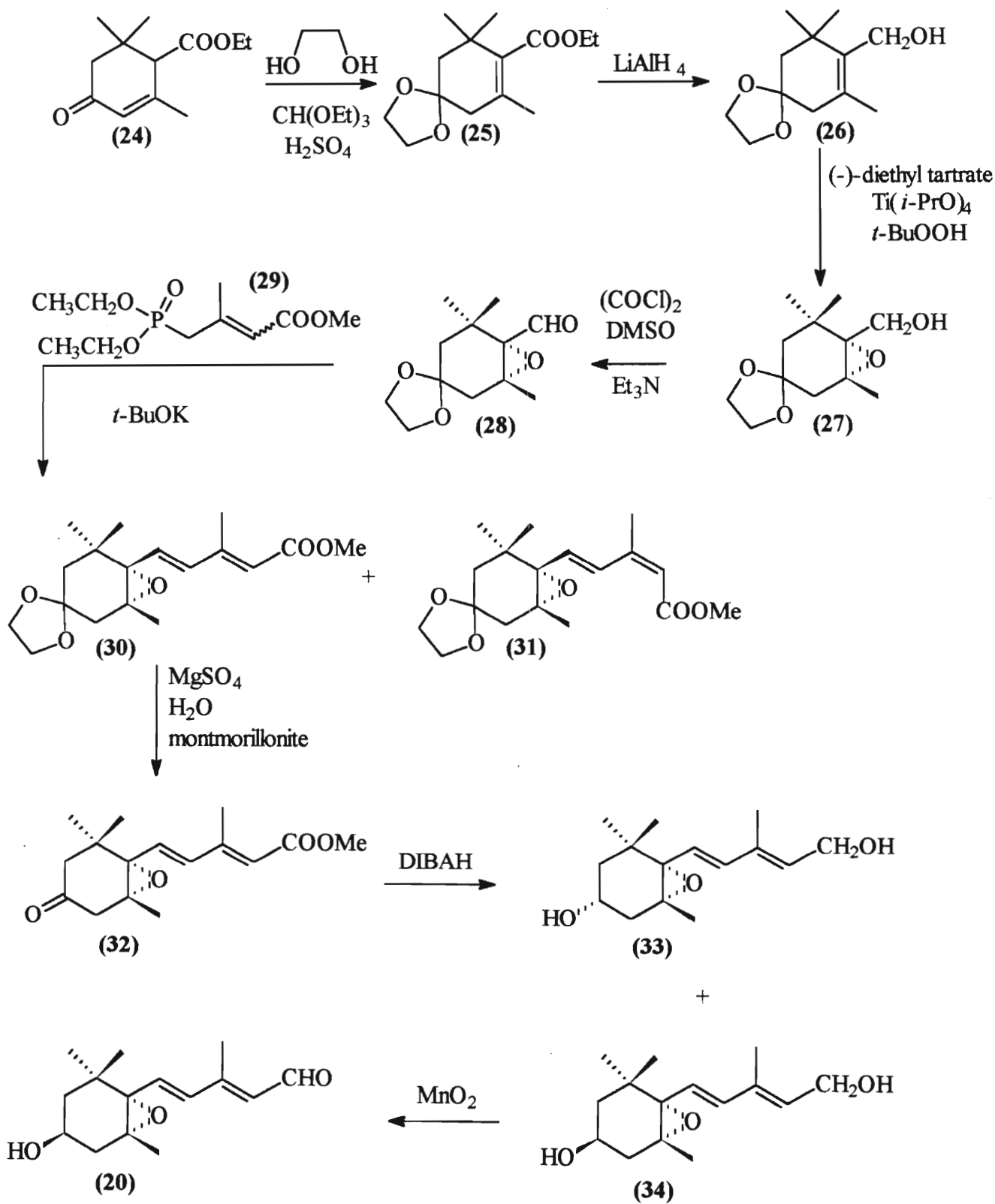
The following synthesis,<sup>100</sup> which was the one chosen for this work, was of special interest to us because the starting materials [mesityl oxide (**22**) and ethyl acetoacetate (**23**)] used were cheap, commercially available, and the synthesis was an industrial process.

**Scheme 3**



Mesityl oxide (**22**) and ethyl acetoacetate (**23**) were subjected to a Robinson annulation by Acemoglu *et al.*<sup>100</sup> using  $\text{ZnCl}_2$  which afforded the  $\text{C}_{10}$ -keto ester (**24**) (**Scheme 3**). Under standard conditions **24** was protected, rearranged and reduced to the allylic alcohol **26** (**Scheme 4**). Sharpless epoxidation of **26** gave in high yield (93%) and high stereoselectivity (97% e.e.) the epoxide **27**. The alcohol was oxidised by a Swern oxidation with oxalyl chloride and dimethyl sulphoxide to the aldehyde **28**. Chain elongation with the  $\text{C}_5$ -phosphonate **17** employing the Horner-Emmons reaction gave the  $\text{C}_{15}$ -esters **30** and **31** in an (*E/Z*)-ratio of 7:1. This reaction however, produced the incorrect isomer as the major product. This, would result in the synthesis of *trans*-Xan, rather than Xan the desired product. From the work carried out by Yamamoto and Oritani<sup>113</sup> it was noted that *trans*-Xan could be converted to Xan by UV radiation. Therefore, *trans*-Xan was synthesized, since a pathway existed for its subsequent conversion to Xan. Deprotection of the ketal and simultaneous reduction of the ketone and ester groups using DIBAH gave a mixture of the diastereomeric diols **33** and **34**, from which the *trans* isomer (**34**) could be crystallised directly. A selective oxidation of **34** with  $\text{MnO}_2$  gave the  $\text{C}_{15}$ -aldehyde, *trans*-Xan (**20**) (**Scheme 4**).<sup>100</sup>

Scheme 4

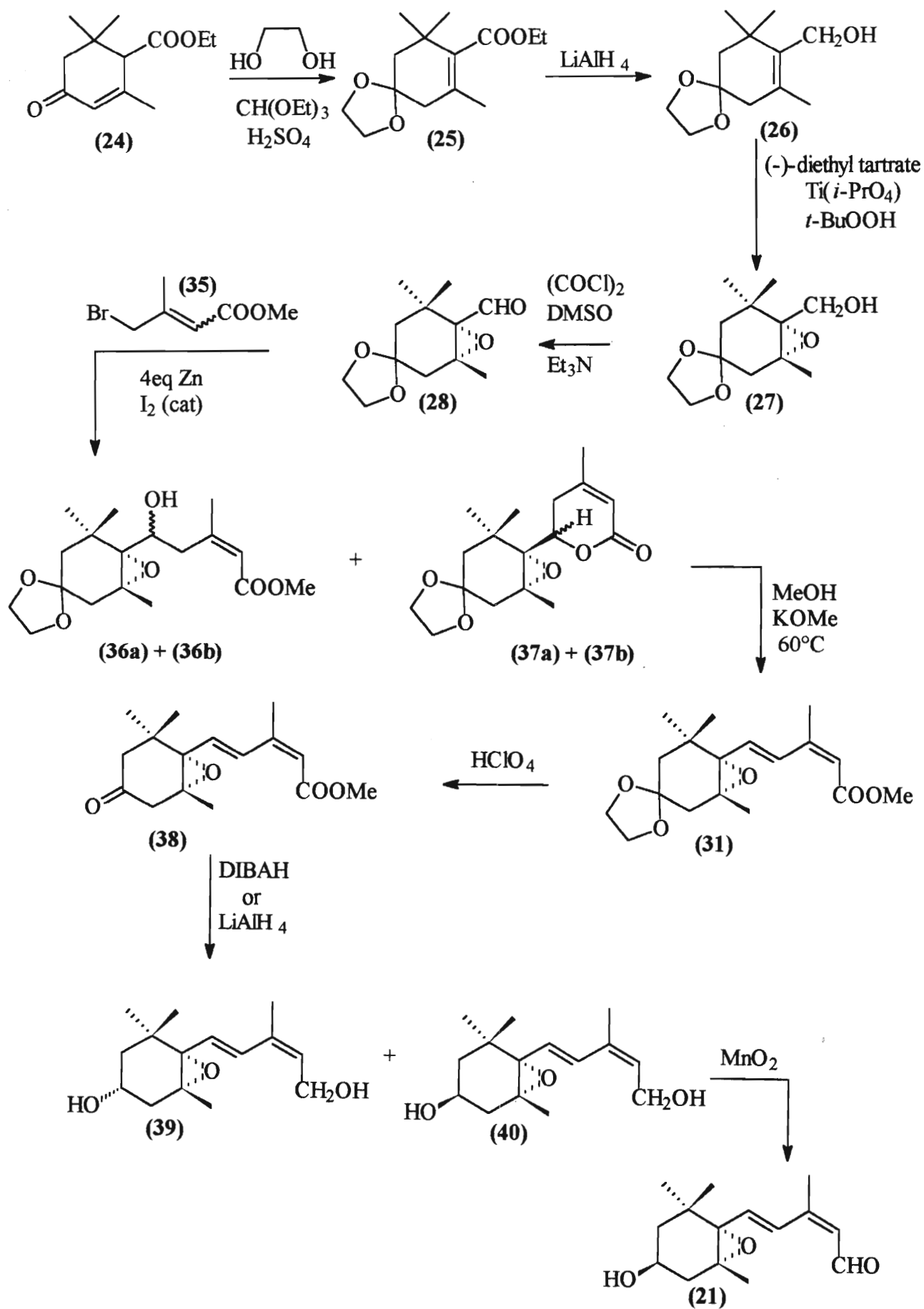


Sakai *et al.*<sup>101</sup> obtained optically active aldehyde **28** from mesityl oxide and ethyl acetoacetate in five steps *via* chiral induction by the Sharpless epoxidation as described above. Their synthetic method was very similar to that shown above in **Scheme 3** with the only major deviation occurring around the chain elongation reaction (**Scheme 5**). Since the stereospecific formation of the (2*Z*,4*E*)-diene structure of the side chain has been a problem in the synthetic work of abscisic acid (**1**),<sup>114,115,116</sup> the Reformatsky reaction was employed to deal with this. From the work carried out by Gedye,<sup>117</sup> Constantino<sup>118</sup> and Kienzle,<sup>103</sup> it was shown that the (2*Z*,4*E*)-diene side chain was constructed with a high degree of stereospecificity from 3-(halomethyl)crotonate and cyclohexenaldehyde. However this reaction had not been applied to epoxy aldehyde derivatives such as **28**, because the Reformatsky reaction was known to react with cyclic ring epoxides.<sup>119</sup>

On the other hand, application of the well-known Wittig-Horner reaction to epoxy aldehydes such as **16** resulted in the reversal of stereospecificity where the (2*E*,3*E*)-isomer was the major product. However, considering the sterically crowded situation around the epoxide ring of the epoxy aldehyde **28**, in which all positions were substituted with bulky groups, it was felt that severe conditions would be required for a reaction to occur at the epoxide ring. For this reason it was argued that Reformatsky conditions could be expected to afford a reaction at the aldehyde group without disturbing the epoxy ring.

Employment of the Reformatsky reaction with **28** and 3-(bromomethyl)crotonate (E:*Z* = 1:1) in the presence of zinc powder by Sakai *et al.*<sup>101</sup> gave a mixture of 4 products which were isolated by column chromatography. They were identified by Sakai *et al.*<sup>101</sup> as hydroxy esters (**36a** and **36b**) and cyclized lactones (**37a** and **37b**) of which all four intermediates gave the single diene (2*Z*,4*E*) product **19** by treatment with alkoxide in high yields, respectively. In the synthesis the reaction products were not separated and treatment of the mixture with methoxide afforded the diene **19** in 92% yield. Treatment of **31** with perchloric acid under controlled conditions<sup>120</sup> gave the deacetalized product **38** exclusively in 86% yield (**Scheme 5**).

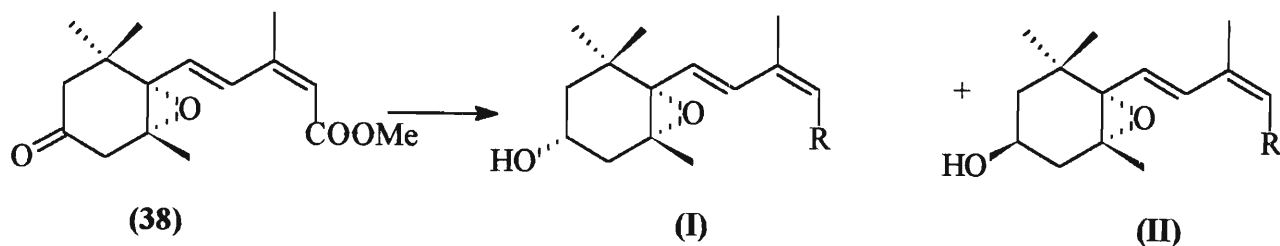
Scheme 5



This synthesis (**Scheme 5**) appeared to be preferable because it employed cheap starting materials and the correct stereochemistry would be obtained to afford Xan, the desired product. In comparison to the synthesis performed by Acemoglu *et al.*<sup>100</sup> this synthesis would be one step shorter due to the fact that 3-(bromomethyl)crotonate would be used rather than the phosphonate in chain elongation, which is derived from 3-(bromomethyl)crotonate. Unfortunately, employment of the reaction of **28** with 3-(bromomethyl)crotonate by us as described by Sakai *et al.*<sup>101</sup> failed completely.

Reduction of the ring carbonyl group to the hydroxy group was examined by Sakai *et al.*<sup>101</sup> under several conditions as summarised in **Table 2**. They found that lithium aluminium hydride and DIBAH were not stereoselective, while sodium borohydride-ceric chloride system gave an  $\alpha$ -OH rich product and K- or L-Selectride provided a  $\beta$ -OH rich product in low yields. The  $\beta$ -OH isomer **40** was converted by Sakai *et al.*<sup>101</sup> to Xan **21** by treatment with  $\text{MnO}_2$ .

**Table 2.**



	Reaction Conditions	R in I and II	I/ II Ratio	% Yield
1	$\text{LiAlH}_4$ , THF, $-25^\circ\text{C}$	$\text{R} = \text{CH}_2\text{OH}$	51 : 49	94
2	DIBAH, toluene, $-78^\circ\text{C}$	$\text{R} = \text{CH}_2\text{OH}$	44 : 56	70
3	$\text{NaBH}_4$ , THF, $-78^\circ\text{C}$	$\text{R} = \text{COOMe}$	64 : 36	67
4	$\text{NaBH}_4$ , THF, $-78^\circ\text{C}$	$\text{R} = \text{COOMe}$	71 : 29	75

5	K-Selectride, THF, -78°C	R = COOMe	23 : 77	54
6	L-Selectride, THF, -78°C	R = COOMe	18 : 82	30

From an analysis of the above results it was decided to perform a simultaneous reduction of the ketone and ester groups of **38**. This was because stereoselective reduction of the ketone followed by reduction with DIBAH or LiAlH<sub>4</sub> would result in a lower yield of the desired isomer being obtained.

## 2. Discussion

### 2.1 Aim

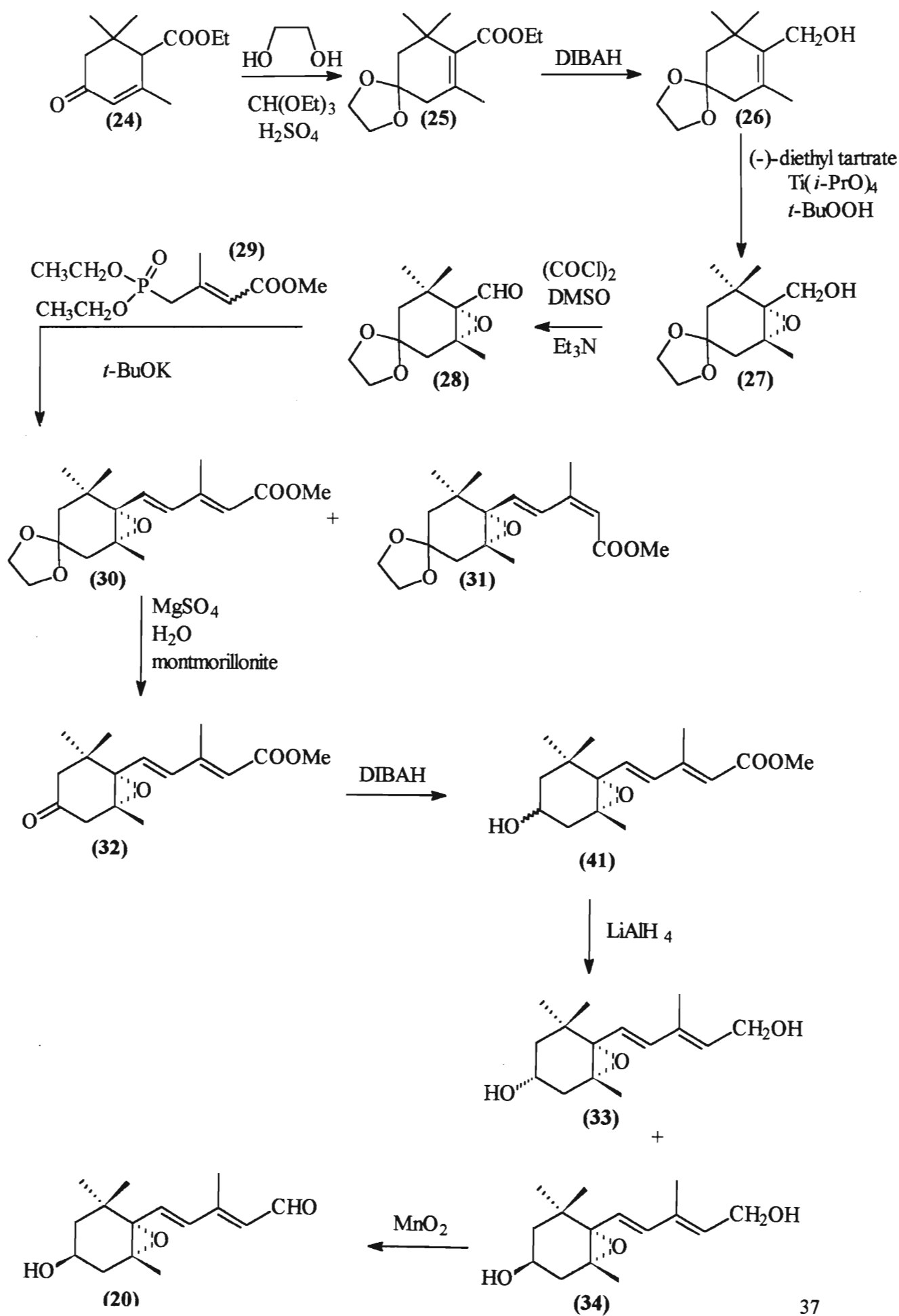
The aim of this project was to synthesise xanthoxal by an efficient and cost effective route as it was needed by another research group at the University of Natal.\* Although much research has been conducted on this subject, horticulturists are still interested in the pathway of ABA biosynthesis in plants as it has yet to be proved conclusively. Since *Citrus* fruit was found to contain ABA<sup>121</sup> and ABA was unequivocally characterised in *Citrus* flavedo<sup>122</sup> containing among the highest ABA concentrations reported for a higher plant tissue,<sup>123</sup> it was envisaged that flavedo might be a useful tissue in which to study aspects of ABA biosynthesis *in vitro*. The accumulated information from investigations carried out by several workers<sup>124,125,126,127,128,129</sup> suggested that cell free extracts of *Citrus sinensis* should contain the necessary enzymes to demonstrate ABA biosynthesis *in vitro*. Employing a cell free system prepared from exocarp of *Citrus sinensis*, research conducted by Cowan and Richardson<sup>82,98,130</sup> confirmed that ABA was derived from a carotenoid. Subsequent ABA biosynthesis was shown to occur via Xan, although the pathway from Xan to ABA has not been confirmed. In order to investigate the conversion of Xan to ABA using the *Citrus* cell free system, Xan (which is not commercially available) was needed.

**Scheme 6** shows the synthetic route employed for the synthesis of *trans*-Xan from mesityl oxide and ethyl acetoacetate (*cf.* **scheme 4** from section 1.4.2). The reagents used in each step are also included in the scheme. Each of the individual steps will be discussed in the following sections.

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\*(Prof A.K. Cowan, Dept. of Horticulture)

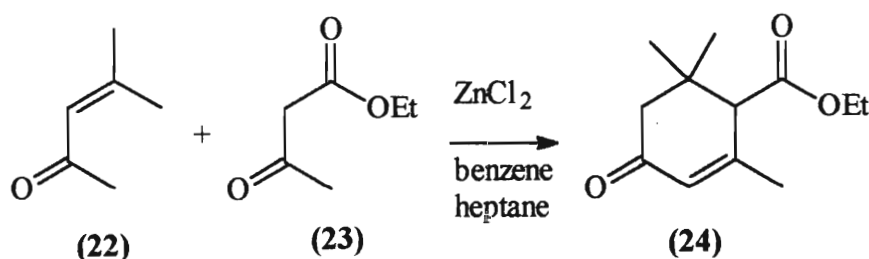
**Scheme 6**



## 2.2 Preparation of the C<sub>10</sub>-ester ketone (24)

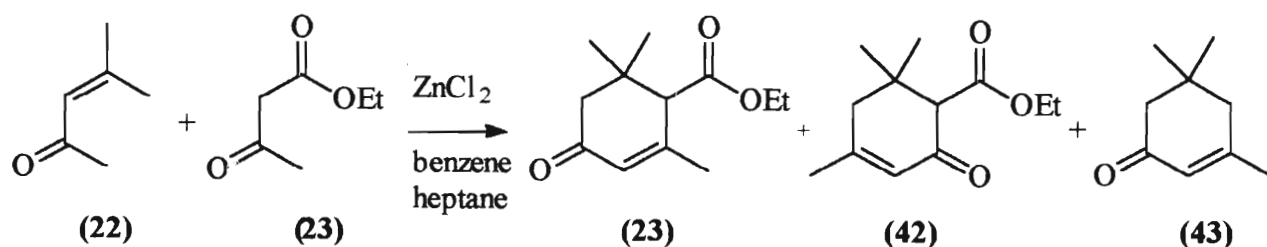
The first step in the synthesis of *trans*-Xan involved the preparation of *ethyl-2,6,6-trimethyl-4-oxo-2-cyclohexene-1-carboxylate* (24) from mesityl oxide (22) and ethyl acetoacetate (23) (scheme 3). An adaptation of the method of Sumatis *et al.*<sup>131</sup> was used which involves the condensation of 22 and 23 using ZnCl<sub>2</sub> as a catalyst.

Scheme 3

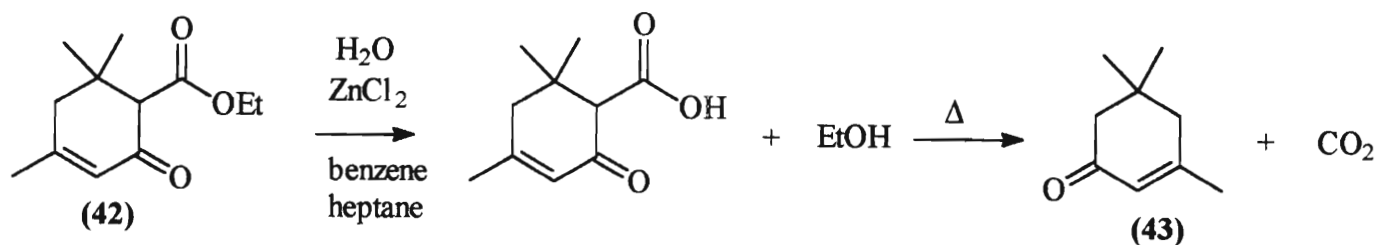


The 1:1 reaction of 22 and 23 in the presence 0.15 eq ZnCl<sub>2</sub> resulted in the formation of two structural isomers, *2,6,6-ethyl-trimethyl-4-oxo-2-cyclohexene-1-one-carboxylate* (12) and *ethyl-4,6,6-trimethyl-2-oxo-3-cyclohexene-1-one-carboxylate ester* (42) (Scheme 7), as well as isophorone (43) which is formed by the hydrolysis of 42 and decarboxylation of the resulting  $\beta$ -keto ester (scheme 8).

Scheme 7

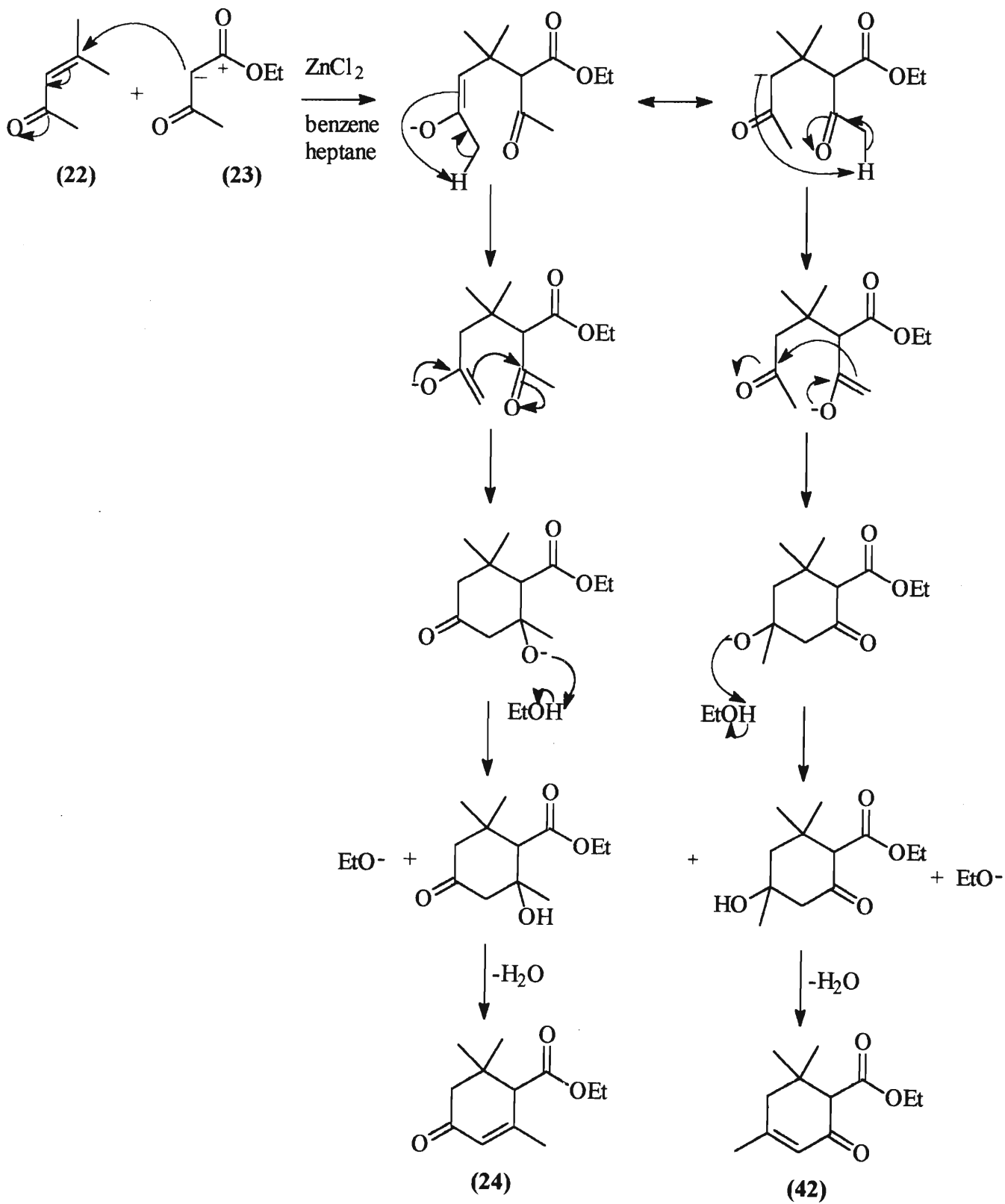


Scheme 8



This was a Robinson annulation type reaction (mechanism shown below in **Scheme 9**), and was repeated under several conditions; the results are summarised in **Table 3**

**Scheme 9**



**Table 3.**

Reaction yields under different conditions

<i>Reactants</i>	<i>No. of Equivalents</i>	<i>Reaction conditions</i>	<i>Product/s</i>	<i>% yield</i>
NaOEt	1	$\Delta$	12/42	1
NaOEt	2	$\Delta$	43	2
NaH	1	$\Delta$	No reaction	
p-TsOH	0.15	$\Delta$	No reaction	
p-TsOH	0.15	r.t.	No reaction	
H <sub>2</sub> SO <sub>4</sub>	Cat.amount	$\Delta$	24/42	1.5
H <sub>2</sub> SO <sub>4</sub> /ZnCl <sub>2</sub>	Cat amount	$\Delta$	24/42	12

From the table above it can be seen that most of the reactions were unsuccessful, while the ones that did work resulted in very low yields.

It was also noted that on replacement of benzene with toluene and heptane with hexane no reaction occurred between **22** and **23** when using zinc chloride as a catalyst. This result indicated the importance of the solvents in this reaction.

Acemoglu *et al.*<sup>100</sup> reported that they carried out the same reaction as reported by Sumatis *et al.*<sup>131</sup> under a nitrogen atmosphere on a kilogram scale. They obtained only two products **24** in 36% yield and **43**. The same reaction as described by Acemoglu *et al.*<sup>100</sup> was carried out by us on a 10 gram scale and the same products were obtained, viz. **24**, **42** and **43** in a similar ratio as reported by Sumatis *et al.*<sup>131</sup> We found no difference between the two reactions.

The authors, Sumatis *et al.*<sup>131</sup> reported a yield of 40% of the two isomers **24** and **42** in the ratio 4:1 while we obtained a comparatively moderate yield of 34%. The slight difference in yield may be due to the distillation procedures employed, where the authors used a packed column while we used a vigreux column. The reported method was performed on a much larger scale, which may be another contributing factor to the

difference in yields. The products were characterised by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy.

### **2.3 Preparation of 4,4-(ethylenedioxy)-2,6,6-trimethyl-1-cyclohexene-1-methanol (26)**

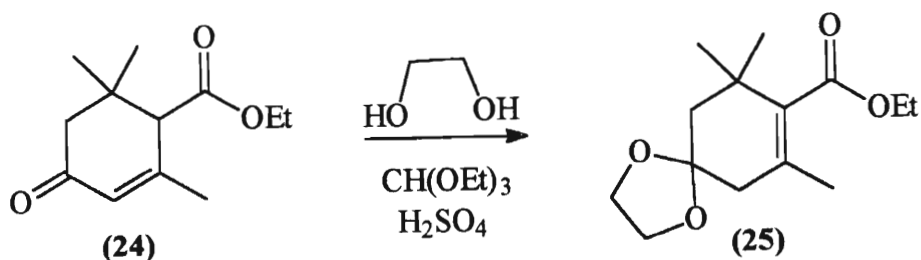
The aim of the next step in the synthesis was to generate the allylic alcohol **26** on which a Sharpless epoxidation could be performed. This could be achieved in two steps by first protecting the ketone from reduction to the alcohol and shifting the double bond from the 2,3 position to the 1,2 position to afford (**25**) and, secondly, by the ester reduction to the primary alcohol (**26**).

#### **2.3.1 Preparation of ethyl 4,4,-(ethylenedioxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxylate (**25**)**

Aldehydes and ketones are extremely versatile compounds for organic synthesis, since they readily undergo nucleophilic attack and can be easily converted into their enolates. However, in polyfunctional molecules, it is often necessary to protect aldehyde and ketone carbonyl groups in order to stop undesirable side reactions during a synthetic sequence, and then remove the protecting group at a later stage. A common protecting group is the ethylene acetal which is easily prepared from the carbonyl compound and ethane-1,2-diol in the presence of an acid catalyst.<sup>132</sup>

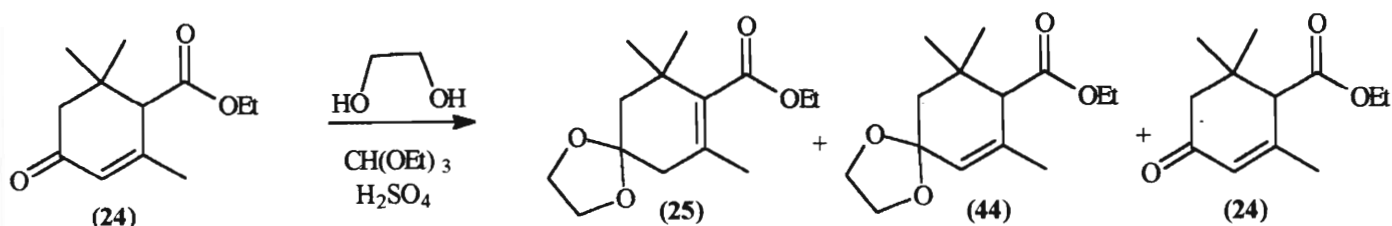
The protection and rearrangement of **24** was performed in one pot where triethyl orthoformate in the presence of  $\text{H}_2\text{SO}_4$  catalyst was employed to shift the position of the double bond, followed by the reaction with ethane-1,2-diol to afford the ketal (**Scheme 10**).

## Scheme 10



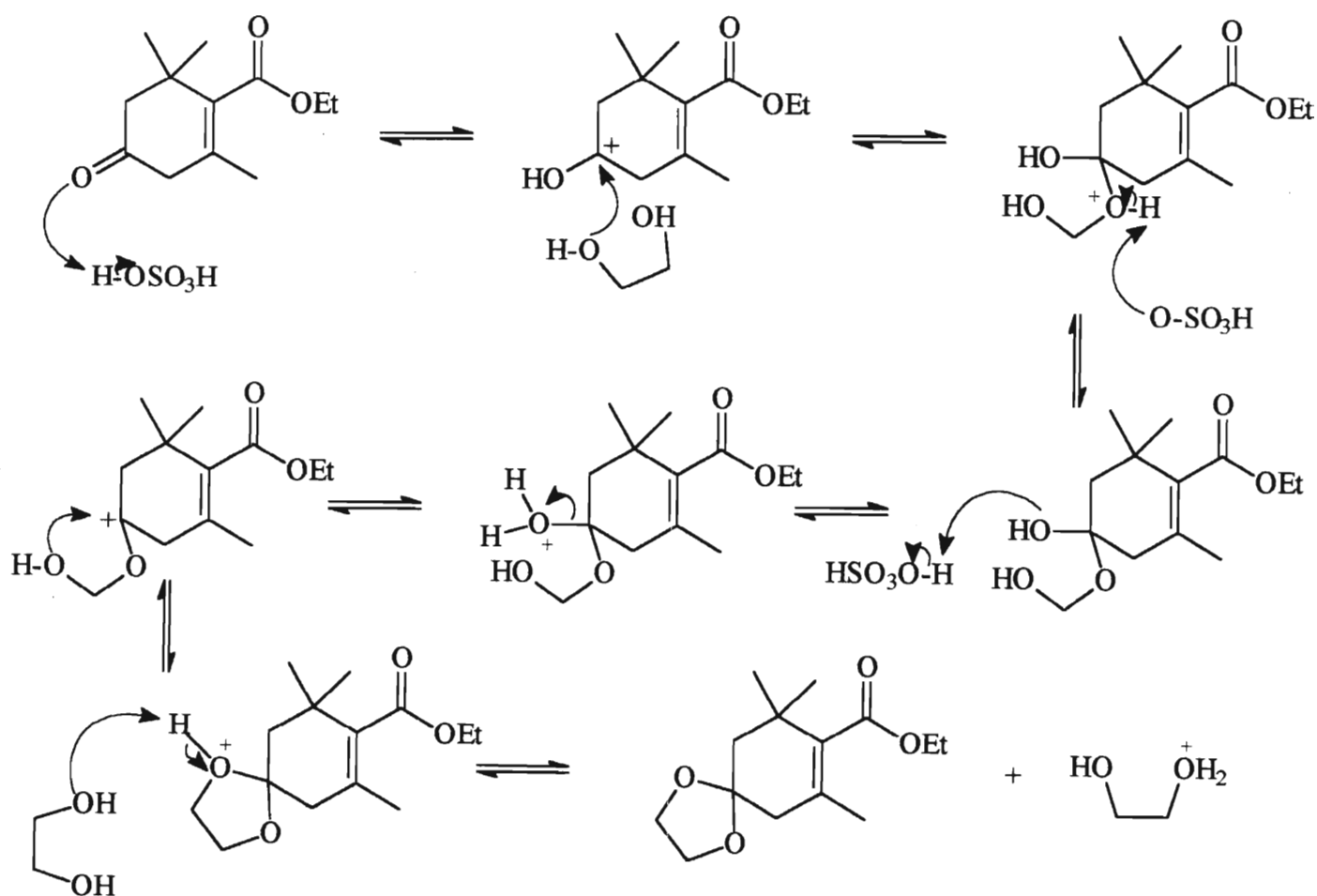
Acemoglu *et al.*<sup>100</sup> reported to have obtained 2 products, viz., **25** and **44** as well as some unreacted starting material **24** in the ratio 61:28:2 (Scheme 11). We obtained **13** in 60% yield (see below).

## Scheme 11



They then treated the mixture with TsOH·H<sub>2</sub>O in the presence of Et<sub>2</sub>O and water and obtained 60% **25**, 29% **24** and 11% unknown products. Separation of the mixture by distillation yielded a distillate containing 24% **25**, 66% **24** and 10% unknown products. This distillate was further treated with triethyl orthoformate, ethane-1,2-diol and H<sub>2</sub>SO<sub>4</sub> which produced a mixture of **25** and **44** which was then partially hydrolysed with *p*-toluenesulfonic acid to **24** and **25**. Overall they obtained **25** in 59% yield. In our employment of this protection rearrangement reaction, a mixture of **24** and **42** were treated with triethyl orthoformate, ethane-1,2-diol and H<sub>2</sub>SO<sub>4</sub>, as reported by the Acemoglu *et al.*<sup>100</sup> The difference in our reaction was that we used twice the number of equivalents of acid compared to that of the authors. We obtained **25** and **42**, which were easily separated by fractional distillation to afford **25** in 60% yield. This yield was comparable to the yield obtained by the authors but only one reaction was performed

### Scheme 12



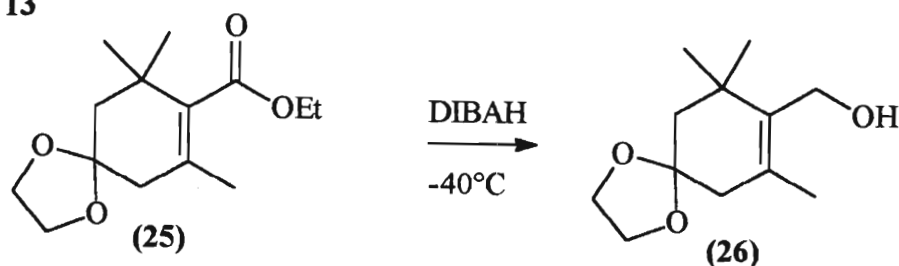
43

### 2.3.2 Reduction of ester 25 to alcohol 26 with DIBAH

Diisobutylaluminium hydride (DIBAH) is an example of a hydride reducing agent having modified activity as a consequence of its bulk. It is often used to convert esters to aldehydes and provides the chemist with a useful midway reagent between  $\text{LiAlH}_4$ , which reduces esters to their alcohols, and  $\text{NaBH}_4$ , which does not reduce esters at all. DIBAH is also capable of reducing a free aldehyde to the alcohol, and will also reduce esters to alcohols. DIBAH has become a common reducing agent, being the reagent of choice in many reductions. DIBAH can also react with aliphatic and aromatic ethers, oxiranes, lactones, amides, lactams and nitriles.<sup>132,133</sup>

DIBAH was used to reduce the ester **25** to the alcohol (**Scheme 13**). This reaction yielded the alcohol exclusively in 74% yield with no further purification being necessary. We obtained a slightly lower yield compared to Acemoglu *et al.*<sup>99</sup> who used  $\text{LiAlH}_4$  and obtained a yield of 75%.

**Scheme 13**



The alcohol (**26**) was characterised by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy. In comparing the  $^1\text{H}$  NMR spectra of **25** and **26**, 3 differences are noted (**Table 4**).

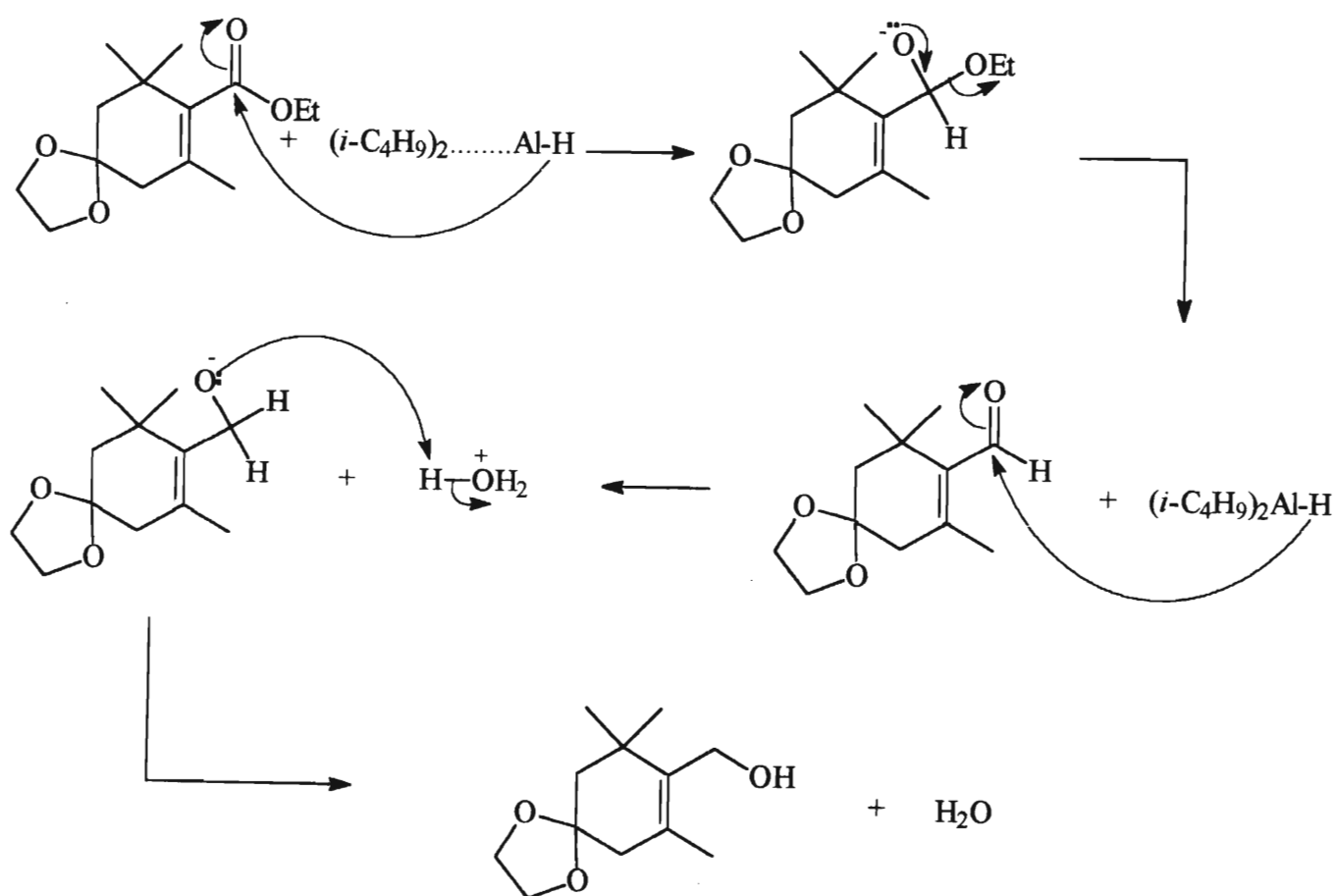
**Table 4**

Compound 25	Compound 26
1.32 (3H, t, $\text{CH}_3 \text{CH}_2\text{O}$ )	No signal
1.70 (3H, s, $\text{CH}_3\text{C}=\text{}$ )	1.80 (3H, s, $\text{CH}_3\text{C}=\text{}$ )
4.28 (2H, q, $\text{CH}_3 \text{CH}_2\text{O}$ )	4.17 (2H, s, $\text{CH}_2\text{OH}$ )

The signal corresponding to the ester  $\text{CH}_3$  protons was absent in **26** due to the loss of the ethyl group in the ester reduction. The signal at 4.17 ppm, appears suitable for  $\text{CH}_2$  protons adjacent to a hydroxyl group. The  $^{13}\text{C}$  spectrum of **26** showed the absence of the signal at *ca* 170 ppm, which corresponds to the carbonyl carbon. The GC/MS analysis showed a clear difference between the ester **25** and alcohol **26**. The alcohol exhibited a lower retention time compared to the ester with a  $\text{M}^+$  peak at  $m/z$  254 compared to an  $\text{M}^+$  peak at  $m/z$  212. This shows clearly the difference of 42 mass units between the ester and the alcohol.

The mechanism<sup>133</sup> for the reduction of the ester to the alcohol is illustrated in **Scheme 14**. The first step involves hydride attack at the carbonyl carbon, which is activated by the prior co-ordination to the electrophilic aluminium and formation of an aluminate salt. The hydride thus replaces alkoxide at the carbonyl group of the ester to give the aldehyde. A second DIBAH molecule would attack the aldehyde to give, after protonation the alcohol.

**Scheme 14**



## 2.4 Sharpless epoxidation of alcohol 26 to epoxide 27

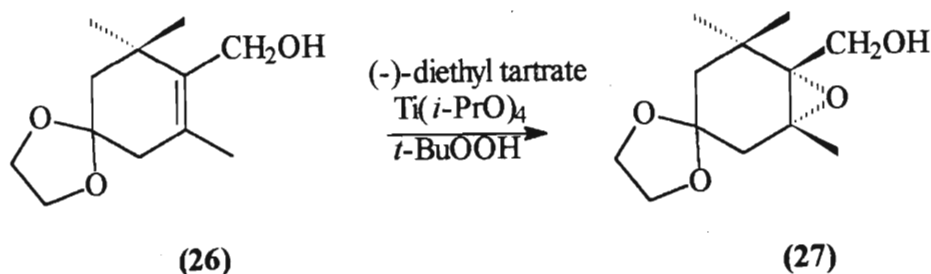
The Sharpless epoxidation is a key reaction in the synthesis of xanthoxal because it allows the generation of an epoxide with the correct stereospecificity which other reagents cannot. The boon with this reagent is that it does not form epoxide mixtures like other reagents would – reagents like magnesium monopero-phthalate (MMPP) and *m*-chloroperoxybenzoic acid (MCPBA). Being an enantioselective reaction producing high yields, and the stereoselectivity removes in most cases the difficult task of separating isomers.

This reaction which was discovered by Professor Barry Sharpless entails the titanium catalysed asymmetric epoxidation of alkenes enantioselectively. The reaction involves the *t*-butylhydroperoxide (TBHP) epoxidation of allylic alcohols in the presence of titanium (IV) tetraisopropoxide using diethyl tartrate as a chiral auxiliary. Although the reaction in its original form required the tartrate and the titanium reagents to be present in stoichiometric amounts, it has now been discovered that only catalytic quantities of these reagents are necessary, making the asymmetric amounts, truly catalytic.<sup>134</sup>

The TBHP Sharpless epoxidation of **26** was run in the presence of catalytic amounts of titanium (IV) isopropoxide, diethyl D-(-)-tartrate and 4 Å molecular sieves (**Scheme 15**). The presence of the molecular sieves is essential for the catalytic reaction since adventitious water seems to destroy the catalyst system. Acemoglu *et al.*<sup>99</sup> and Sakai *et al.*<sup>100</sup> both reported methods for the Sharpless epoxidation of **26** to **27**. The difference in the methods was the temperature at which the reaction was carried out; the former used -60°C while the latter -20°C. We found that at -60°C no reaction took place, and on warming the reaction mixture to -20°C the reaction proceeded slowly. Acemoglu *et al.*<sup>99</sup> and Sakai *et al.*<sup>100</sup> reported yields of 93% and 75% with e.e.'s 97% and 90% respectively. We obtained a favourable yield of 99%.

The epoxide **27** characterised by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy. Both spectral data compare favourably with those in the literature.<sup>99,100</sup> In the  $^{13}\text{C}$  spectrum the disappearance of

#### Scheme 15



the alkene carbon signals at 130.20 ppm and at 136.92 ppm are clear indications of saturation at C-1 and C-2.

### 2.5 Swern oxidation of epoxy alcohol **27** to aldehyde **28**

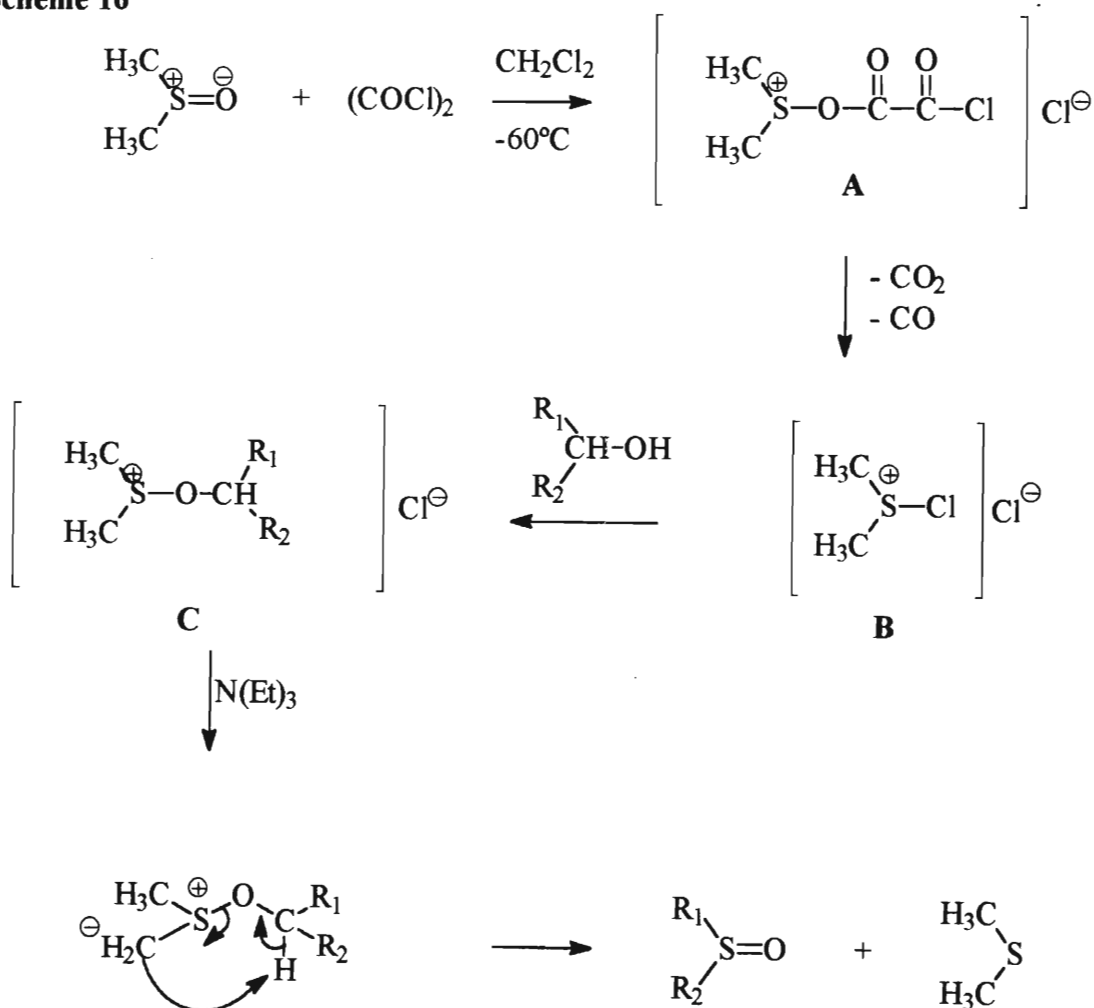
This step in the synthesis involves a Swern oxidation of the epoxy alcohol to the epoxy aldehyde, which is performed in order to prepare the molecule for chain elongation to be carried out in the following step. Since the Horner-Emmons reaction is a Wittig type reaction, an electrophilic centre is needed as well as a nucleophilic side chain, which takes the form of a phosphonate.

Oxidation of alcohols to their corresponding carbonyl compounds is a very important process in organic synthesis, and many reagents have been developed for this transformation. The development of selective and efficient reagents for conversion, in the presence of other oxidizable functional groups, has been of major importance to organic chemists. Since many preparative methods are accompanied by many restrictions, new, mild, and selective procedures seem more attractive. A major breakthrough was the development of dimethyl sulfoxide (DMSO) “activated” by

oxalyl chloride at low temperatures, reacting rapidly to give alkoxyulfonium salts, which are converted to carbonyl compounds in high yields on addition of triethylamine.<sup>135,136</sup>

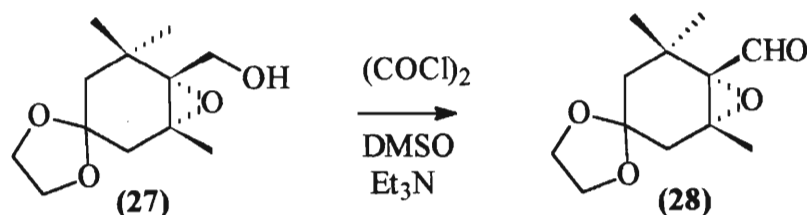
The epoxy alcohol **27** was oxidised using DMSO, which was “activated” by the most efficient and generally useful DMSO “activator”, oxalyl chloride. The mechanism in **Scheme 16** illustrates how oxalyl chloride reacts with DMSO. Oxalyl chloride reacts violently and exothermically with DMSO at room temperature; therefore successful activation requires the use of low temperatures ( $-60^{\circ}\text{C}$ ) to form the intermediate **B**, which is obtained from the spontaneous loss of carbon dioxide and carbon monoxide from **A**. The key intermediate, the alkoxyulfonium salt **C** is formed by the nucleophilic substitution of chloride by an alcohol. Oxidation occurs *via* ylide **D**, which collapses by an intramolecular cyclic system thus producing the desired carbonyl compound and dimethyl sulfide.

**Scheme 16**



The epoxy alcohol (**27**) was oxidised to the aldehyde (**16**) (**Scheme 17**) in a crude yield of 90%. The aldehyde was not purified further due to it being unstable and decomposing when purification by Kugelrohr distillation was attempted.

**Scheme 17**



In comparing both the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of the alcohol **27** and the aldehyde **28** there is only a slight difference in the chemical shifts and coupling constants. The aldehyde proton was clearly visible in the  $^1\text{H}$  NMR spectrum at 9.79 ppm as was the aldehyde carbon at 200.8 ppm in the  $^{13}\text{C}$  NMR spectrum.

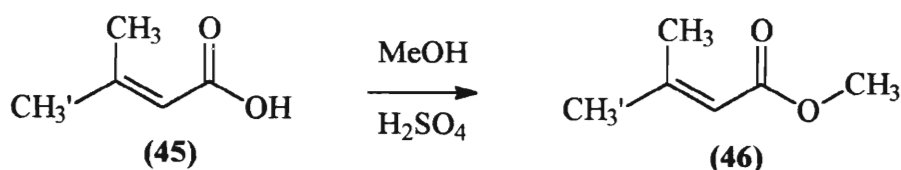
## 2.6 Preparation of the C<sub>5</sub>-phosphonate side chain (17)

Preparation of the side chain dimethyl 3-methoxycarbonyl-2-methylprop-2-enyl phosphonate (29) involves 3 steps. The phosphonate can be easily prepared from methyl 3,3-dimethylacrylate, which is commercially available. Due to the cost of methyl 3,3-dimethylacrylate, the ester was prepared from the much cheaper acid 3,3-dimethylacrylic acid.

### 2.6.1 Esterification of 3,3-dimethylacrylic acid

When a carboxylic acid is treated with a large excess of alcohol in the presence of a strong acid catalyst, an ester is formed. The ester (46) was prepared employing standard procedures of acid + alcohol = ester + water. Anazole and Hirsch<sup>137</sup> and Steven *et al.*<sup>138</sup> reported the use of different acids with different alcohols. The method we used involved reacting the acid with methanol in a catalytic quantity of H<sub>2</sub>SO<sub>4</sub> which yielded the methyl ester in a moderate yield of 58% (Scheme 18).

Scheme 18



The ester (46) was characterised by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. The methoxy proton signal was clearly visible in the <sup>1</sup>H NMR spectrum at 3.68 ppm, as was the methoxy carbon atom at 50.77 ppm in the <sup>13</sup>C NMR. In the mechanism, the carboxylic acid is first protonated. Having a carbocation character, the protonated species is then

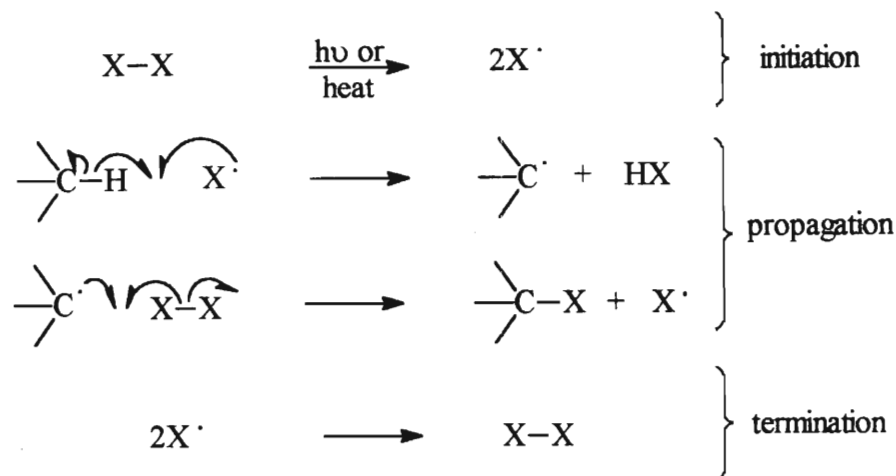
attacked by the alcohol forming a tetrahedral intermediate after loss of a proton. The tetrahedral intermediate reacts further by proton transfer, loss of water and deprotonation to give the ester.

### 2.6.2 Preparation of Methyl 4-bromosenecioate

Since the object was to synthesize a side chain containing an allylic phosphonate, an allylic hydrogen from the methyl 3,3-dimethylacrylate needed to be replaced by phosphite. There was no direct method of achieving this goal, and thus a bromination was carried out. The bromination reaction involves the abstraction of an allylic hydrogen atom followed by replacement with a bromine atom. Allylic and benzylic alkyl halides are considerably more reactive in  $S_N1$  reactions than their non-allylic counterparts thus making the bromide ion a good leaving group, which can then be easily replaced by phosphite.

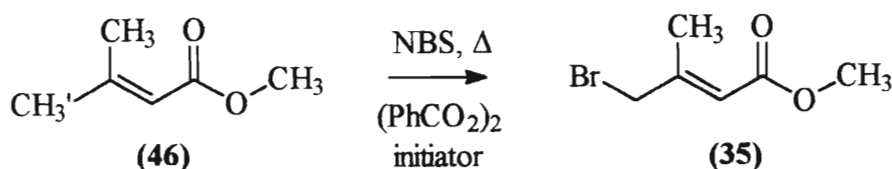
The bromination reaction occurs by free radical substitution. Free radical substitution of hydrogen in organic substrates follows the mechanism of a chain reaction which consists of three phases, viz., *initiation*, *propagation* and *termination* (Scheme 19).<sup>132</sup> The utilisation of *N*-bromosuccinimide (NBS), as a bromine source, and benzoyl peroxide, as the initiator, is a convenient procedure for generating bromine radicals. Heating benzoyl peroxide causes homolytic cleavage of the labile peroxide linkage, producing radicals, which react with NBS, breaking the weak N-Br bond to generate bromine radicals. The HBr generated by allylic hydrogen abstraction from the methyl 3,3-dimethylacrylate reacts with more NBS to form molecular bromine, which in turn combines with the allyl radical to furnish the product and another bromine radical. Thus, the bromine atom functions as a chain carrier.<sup>132</sup>

## Scheme 19



The allylic bromide, methyl 4-bromosenecioate (**35**) was formed in 39% yield (**Scheme 20**). This is much lower than the yield of 60% reported by Ziegler *et al.*<sup>139</sup> in 1942. Since a 0.25 equivalents of NBS was used, more starting material than product was recovered making the reaction tedious; thus several reactions had to be performed in order to generate enough allylic bromide. Several different bromination methods were investigated. It was found that the use of an equal number of equivalents of NBS and methyl 3,3-dimethylacrylate (**46**) with the aid of an initiator yielded a mixture of bromides, which were separated to give the desired product. The bromide (**35**) was characterised by <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopy. The allylic CH<sub>2</sub> protons adjacent to the bromine was evidenced by a two proton singlet at 3.95 ppm in the <sup>1</sup>H NMR spectrum, and the CH<sub>2</sub> carbon resonated at 38.19 ppm in the <sup>13</sup>C NMR spectrum.

## Scheme 20

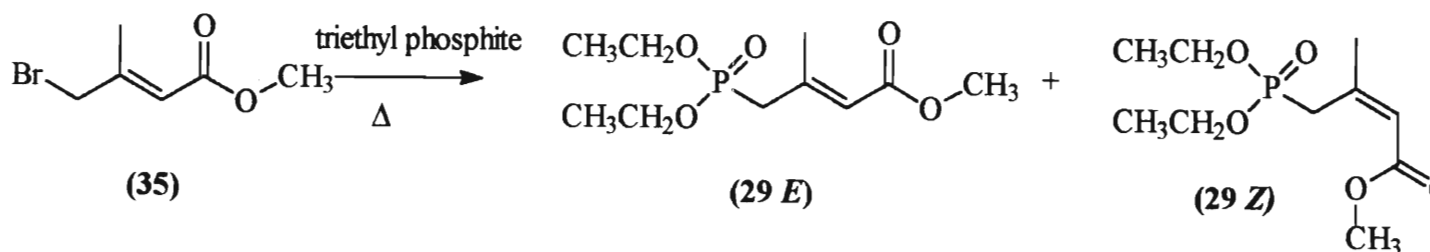


### 2.6.3 Preparation of Diethyl 3-methoxycarbonyl-2-methylprop-2-enyl phosphonate (17)

Phosphonates (Horner-Emmons reagents) are the most commonly used phosphoryl-stabilized carbanions.<sup>140</sup> They are more nucleophilic than the corresponding phosphonium ylides. An additional advantage of these reagents is that the by-products of the alkenation (Horner-Emmons reaction) are water-soluble. A disadvantage of the phosphonate reagent is that a stabilising group such as a carboxyl derivative must be present in the  $\alpha$ -position, unless a two step addition and elimination strategy is employed.

The phosphonate 17 was prepared from methyl 4-bromosene-2-carboxylate (35) and triethyl phosphite in 74% yield (Scheme 21) which was slightly lower than the 79% reported by Davies *et al.*<sup>141</sup> GC-MS and NMR spectroscopy showed the presence of E and Z isomers in the ratio of 3:1. In comparison to the E:Z ratio of 3:2 reported by Davies *et al.*<sup>141</sup> we obtained a slightly higher ratio of the E isomer. This could be due to the difference in reaction temperature since Davies *et al.*<sup>141</sup> reported heating the reaction mixture to 150°C while the internal temperature of our reaction mixture did not rise above 120°C at which it was refluxing.

## Scheme 21

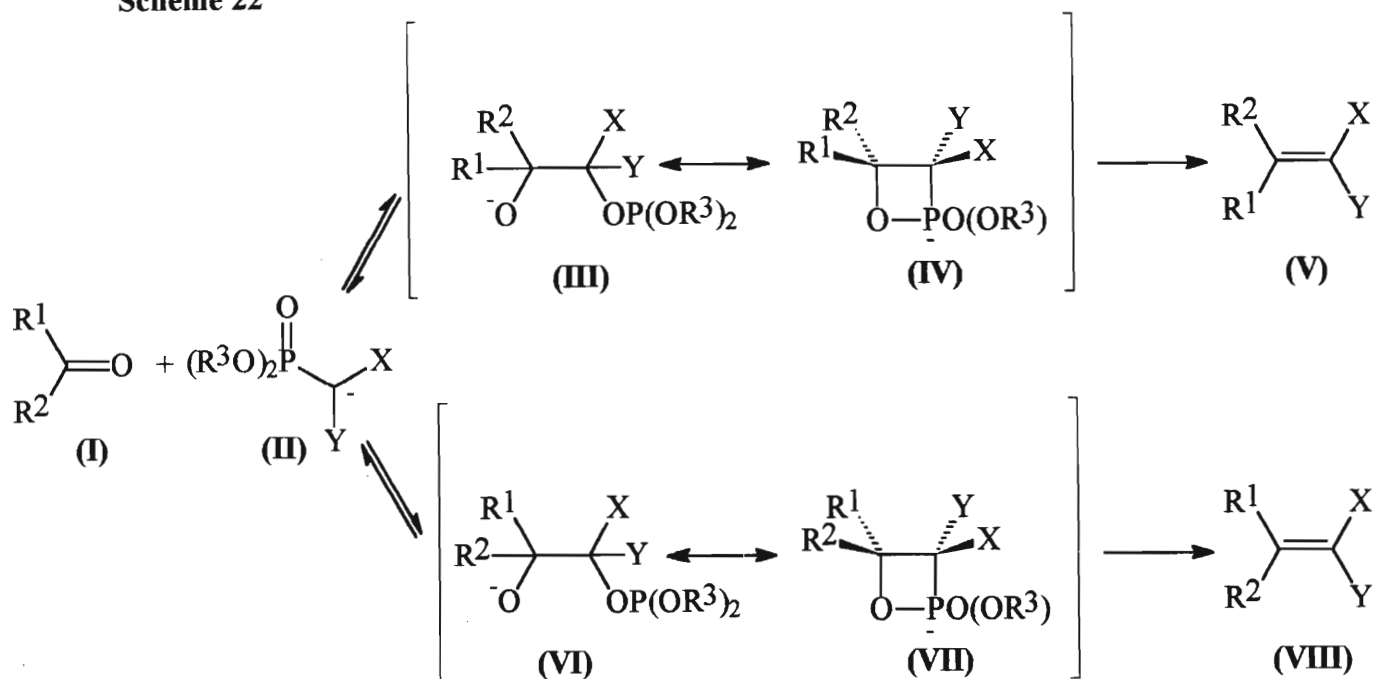


The different signals due to the *E* and *Z* isomers were evident in both the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. The methyl proton signals of the *Z*- and *E*-isomers were clearly visible in the  $^1\text{H}$  NMR spectrum at 2.07 and 2.32 ppm respectively as were the  $\text{CH}_3$  carbon signals of the *E*- and *Z*-isomers at 20.07 and 26.18 ppm respectively in the  $^{13}\text{C}$  NMR spectrum. The phosphonate  $\text{CH}_3$  and  $\text{CH}_2$  proton signals were also visible in the  $^1\text{H}$  NMR spectrum at 1.33 and 4.12 ppm respectively as were their corresponding carbon signals at 16.42 and 62.28 ppm respectively in the  $^{13}\text{C}$  NMR spectrum.

## **2.7 Chain elongation of the aldehyde 28 by a Horner-Emmons reaction**

The mechanism of phosphonate anion (**II**) addition to carbonyl derivatives is similar to phosphonium ylide addition (**Scheme 22**); however, there are several notable features to these anion additions that distinguish the reactions from those of the classical Wittig. The addition of the anion gives a mixture of the *erythro* (**III** and **IV**) and *threo* (**VI** and **VII**)  $\beta$ -hydroxyphosphates. In the case of phosphine oxides, the initial oxyanion intermediates may be trapped. The anion intermediates decompose by a *syn* elimination of phosphate or phosphinate to give the alkene. The elimination is stereospecific, with *erythro* isomer producing the (*Z*)-alkene (**V**) and the *threo* addition adduct producing the (*E*)-alkene (**VIII**). The ratio of (*E*) and (*Z*)-alkenes is dependent on the initial ratio of *erythro* and *threo* adducts formed, as well as their ability to equilibrate.<sup>142</sup>

Scheme 22



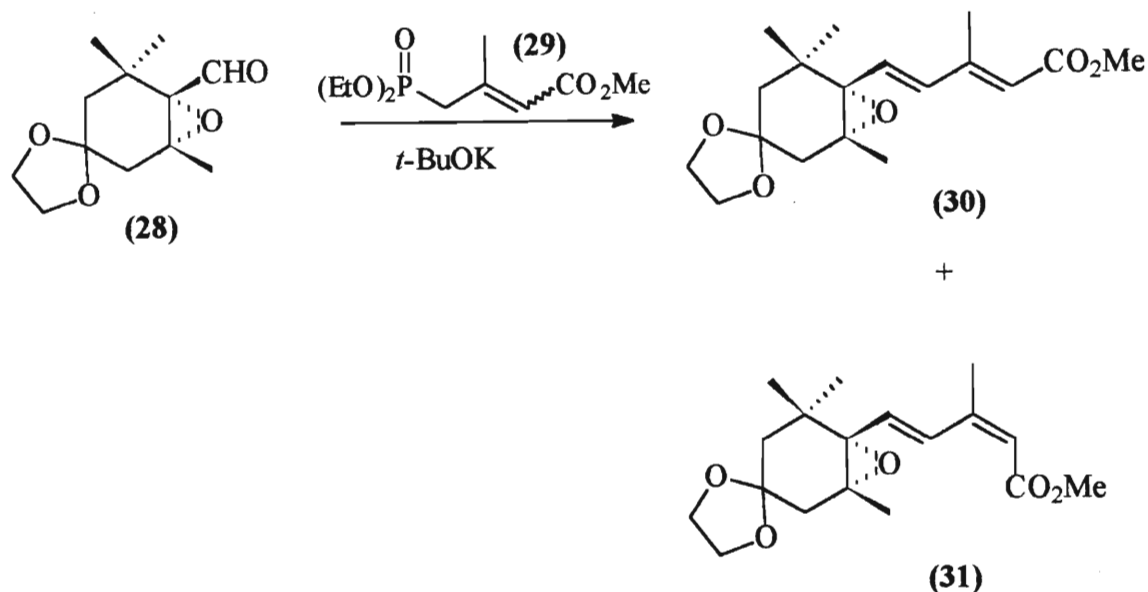
Chain elongation of **28** with the C<sub>5</sub>-phosphonate **29** by a Horner-Emmons reaction resulted in the C<sub>15</sub> esters **30** and **31** in an (*E*:*Z*) ratio of 6.4:1 with a yield of 54% (Scheme 23). The yield was lower than that reported in the literature while the ratio of the isomers was similar.<sup>100</sup> The *Z* isomer **31**, is an ideal compound from which xanthoxal, the desired compound could be synthesized. Unfortunately, since the yield of **19** was small, not enough compound, was generated to complete the synthesis. Thus the *E* isomer **31** was used to produce *trans*-xanthoxal which could be converted to xanthoxin at a later stage. Both the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra agreed with the published literature values.<sup>100</sup> The differences in compounds **30** and **31** are clearly visible in the <sup>1</sup>H spectra as shown in the table below.

Compound 30	Compound 31
6.33(2H, d, <i>J</i> =1.1, <u>HC</u> (5)); <u>HC</u> (4))	6.29, 7.63 (2H, <i>J</i> <sub>AB</sub> =16, <u>HC</u> (5)); <u>HC</u> (4))

The signals representing the protons at C-4 and C-5 of **30** (the *E* isomer) are unsplit and further upfield compared to the signals of the corresponding protons of **31** that are

split and much further downfield as a result of deshielding by the ester. An inset has been added to the spectrum of **18** in order to calculate the coupling constant of the signal corresponding to the equatorial proton at C-5'.

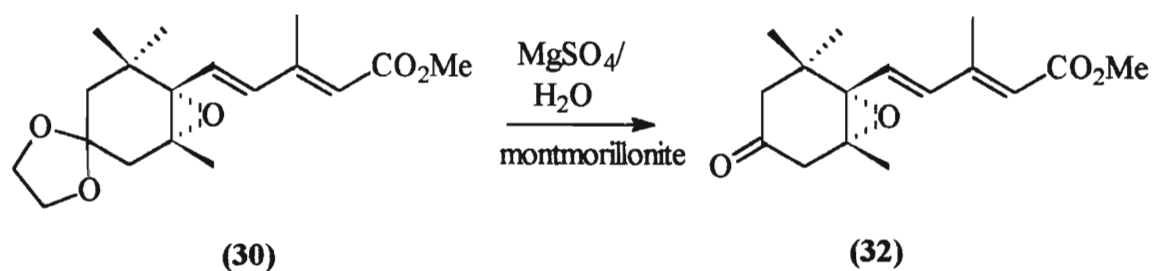
**Scheme 23**



## 2.8 Deprotection of the ketone (18)

The acetal was removed by acid catalysed hydrolysis to produce the ketone **(20)** in 81% yield (**Scheme 24**). Because ketal formation is reversible, acetals in the presence of acid and excess water are rapidly transformed back into their corresponding carbonyl compounds. By the principle of microscopic reversibility, the mechanism of acetal hydrolysis is the reverse of acetal formation.

**Scheme 24**

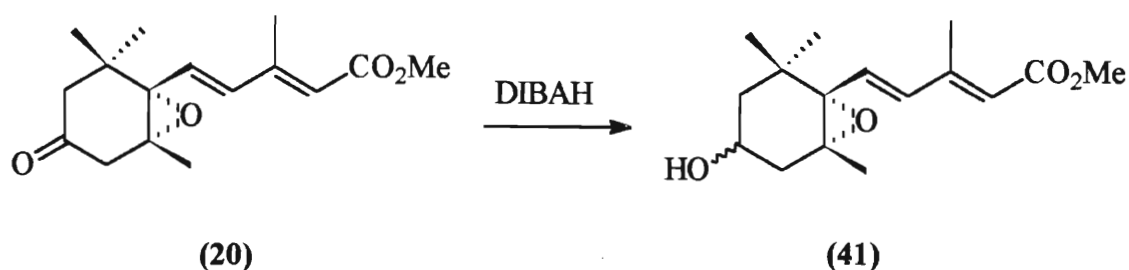


The compound **32** was characterised by both  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopy. The absence of a multiplet at *ca.* 3.9 ppm in the  $^1\text{H}$  NMR spectrum, (due to the  $\text{CH}_2$  protons of the ketal) and the presence of a carbonyl carbon signal at 207.21 in the  $^{13}\text{C}$  NMR spectrum both indicated that the deprotection had been successful.

### **2.9.1 Simultaneous reduction of the ester and ketone groups to their respective alcohols**

The simultaneous reduction of both the ester and ketone groups using DIBAH was unsuccessful. DIBAH reduced only the ketone to the corresponding alcohol with no trace of any diol being formed. This was a surprise to us as both Acemoglu *et al.*<sup>100</sup> and Sakai *et al.*<sup>101</sup> had reported that they were able to perform this operation. We also attempted to reduce the ester (**32**) to the aldehyde using DIBAH, which would have resulted in the final product, thus saving two steps in the synthesis, but this was also not achieved. The alcohol (**41**) was formed in 82% yield (**Scheme 25**). Compound **41** was characterised by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopy. From the  $^{13}\text{C}$  NMR spectrum it was apparent that a mixture of diastereomeric alcohols which was expected, was present. The mixture was not separable by chromatography. Both  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra compared favourably with the published literature values.<sup>100</sup> The absence of the carbonyl carbon signal occurring at *ca.* 207.21 in the  $^{13}\text{C}$  NMR spectrum was a clear indication that the keto group was no longer present.

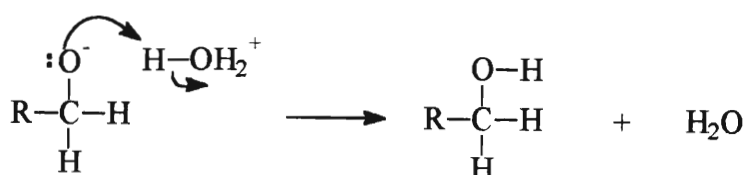
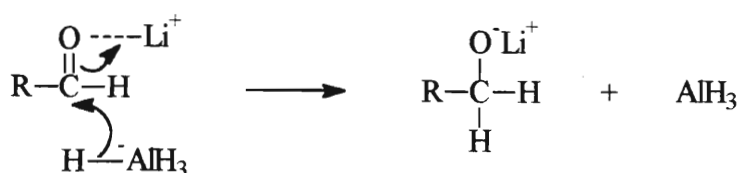
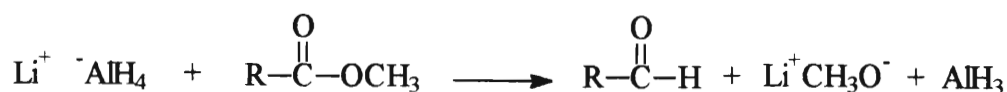
**Scheme 25**



### 2.9.2 Reduction of the ester (41) with LiAlH<sub>4</sub> to the alcohol

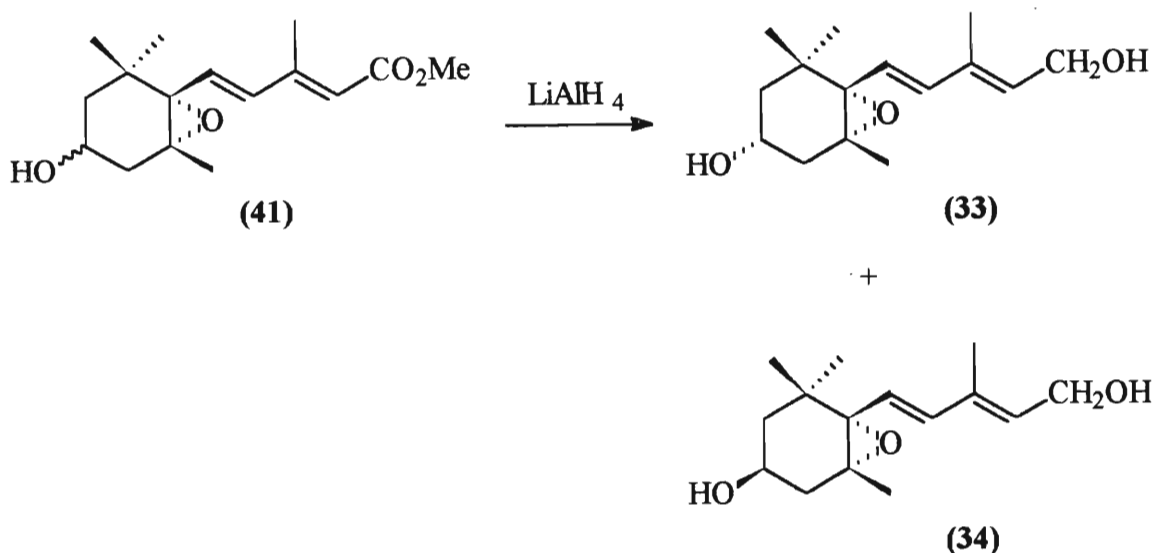
Hydride transfer reagents are commonly used as reducing agents in organic synthesis. The two most frequently used are lithium aluminium hydride (LiAlH<sub>4</sub>) and sodium borohydride (NaBH<sub>4</sub>). Although both these reagents can be regarded as a source of nucleophilic hydride, their reducing powers are quite different; where LiAlH<sub>4</sub> is a much more powerful reductant and reduces most functional groups which contain a polarized multiple bond. As a consequence of this greater reactivity it is much less selective than NaBH<sub>4</sub>. The reducing power of LiAlH<sub>4</sub> can be attenuated by replacing one or more of the hydrogen atoms by an alkoxy group. LiAlH<sub>4</sub> finds particular application in the reduction of carboxylic acid derivatives to alcohols. Reduction of esters with this reagent, like reduction of carboxylic acids, gives primary alcohols. As with all hydride reductions of carbonyl compounds, the key step involves transfer of hydride to the electrophilic carbonyl carbon. The general mechanism (**Scheme 26**) for ester reduction involves the replacement of alkoxide at the carbonyl carbon of the ester with the active nucleophile H<sup>-</sup> delivered from <sup>-</sup>AlH<sub>4</sub> to give an aldehyde. The aldehyde then reacts rapidly with LiAlH<sub>4</sub> to give after protonation the alcohol.

**Scheme 26**



Reduction of the ester (**41**) with  $\text{LiAlH}_4$  gave a mixture of the diastereomeric diols **33** and **34** in a ratio of 1: 15 in 39% overall yield (**Scheme 27**). Both the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra compared favourably with the published literature values.<sup>100</sup> The methyl proton signal of the ester at 3.71 ppm was not present in the  $^1\text{H}$  NMR spectrum and the carbonyl resonance at 167.51 ppm in the  $^{13}\text{C}$  NMR spectrum had disappeared. The  $\text{CH}_2$  proton signal of the primary alcohol was clearly visible in the  $^1\text{H}$  NMR spectrum at 4.30 ppm as was the  $\text{CH}_2$  carbon signal of the primary alcohol at 59.31 ppm in the  $^{13}\text{C}$  NMR spectrum

**Scheme 27**



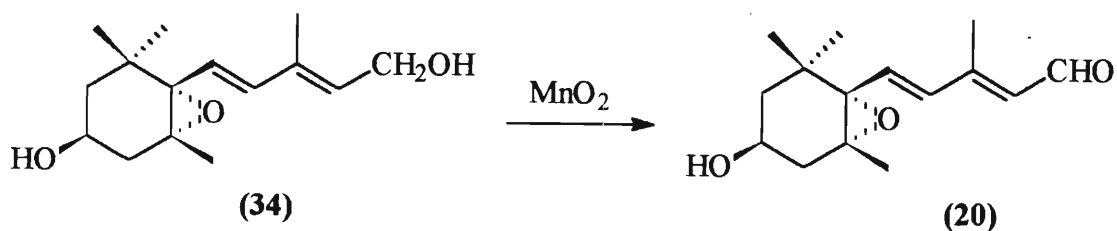
### 2.9.3 Oxidation of the diol **22** to the aldehyde **23**

The oxidation of an alcohol to a carbonyl compound is a fundamental reaction, which is encountered at all levels of organic synthesis. As might be expected, there are numerous methods available for carrying out this transformation. The usefulness of a reagent is critically dependent upon its selectivity, mildness and availability. Oxidation of primary alcohols to aldehydes in the presence of other groups which themselves are

easily oxidised is possible, but the extent of chemoselectivity is dependent on the reagent. Generally, benzylic and allylic alcohols are more easily oxidised than alcohols in which the hydroxy group is not 'activated' in this way. The selective oxidation of diols in which one or both of the hydroxy groups are allylic has been reported on many occasions. A classical reagent, which can perform this operation, is manganese dioxide,<sup>143,144</sup> which is of value not only for its inherent chemoselectivity, but also because of the mild conditions under which oxidation occurs.

Selective oxidation of the diol (**34**) resulted in the aldehyde (**20**), (*trans*-xanthoxal) in a moderate yield of 74% (**Scheme 28**). The <sup>1</sup>H NMR spectrum compared favourably with the literature.<sup>99</sup> The aldehyde proton signal at 10.13 ppm was clearly visible in the <sup>1</sup>H NMR spectrum.

**Scheme 28**



## 2.10 Future investigations

With organic chemistry being such a wide and diverse field, there are almost always alternative procedures that may be used instead of the chosen one. Every individual must decide on the most appropriate cost-effective methods for the type of synthesis required.

The reactions described above can be further investigated in respect to (a) using a different starting material or optimisation of the yield of the current starting material **24**, (b) The process of changing reaction conditions in order to obtain better stereoselectivity removing the painstaking task of separating isomers, and (c) attempting alternative pathways which may result in a better overall yield.

The progress towards the synthesis of xanthoxal has highlighted the difficulty in obtaining the isomer **31**, which is the desired precursor to xanthoxal. This may be due the isomer **30** being the more stable of the two. The Reformatsky reaction conditions reported by Sakai *et al.*<sup>101</sup> which produces the isomer **31** exclusively needs further investigation in order to determine reason for the failure in our attempt.

## **2.11 Conclusion**

To obtain the final product, xanthoxal, would be the desired conclusion of this project. We have synthesised the isomer, *trans*-xanthoxal, which can be converted in xanthoxal by UV radiation.

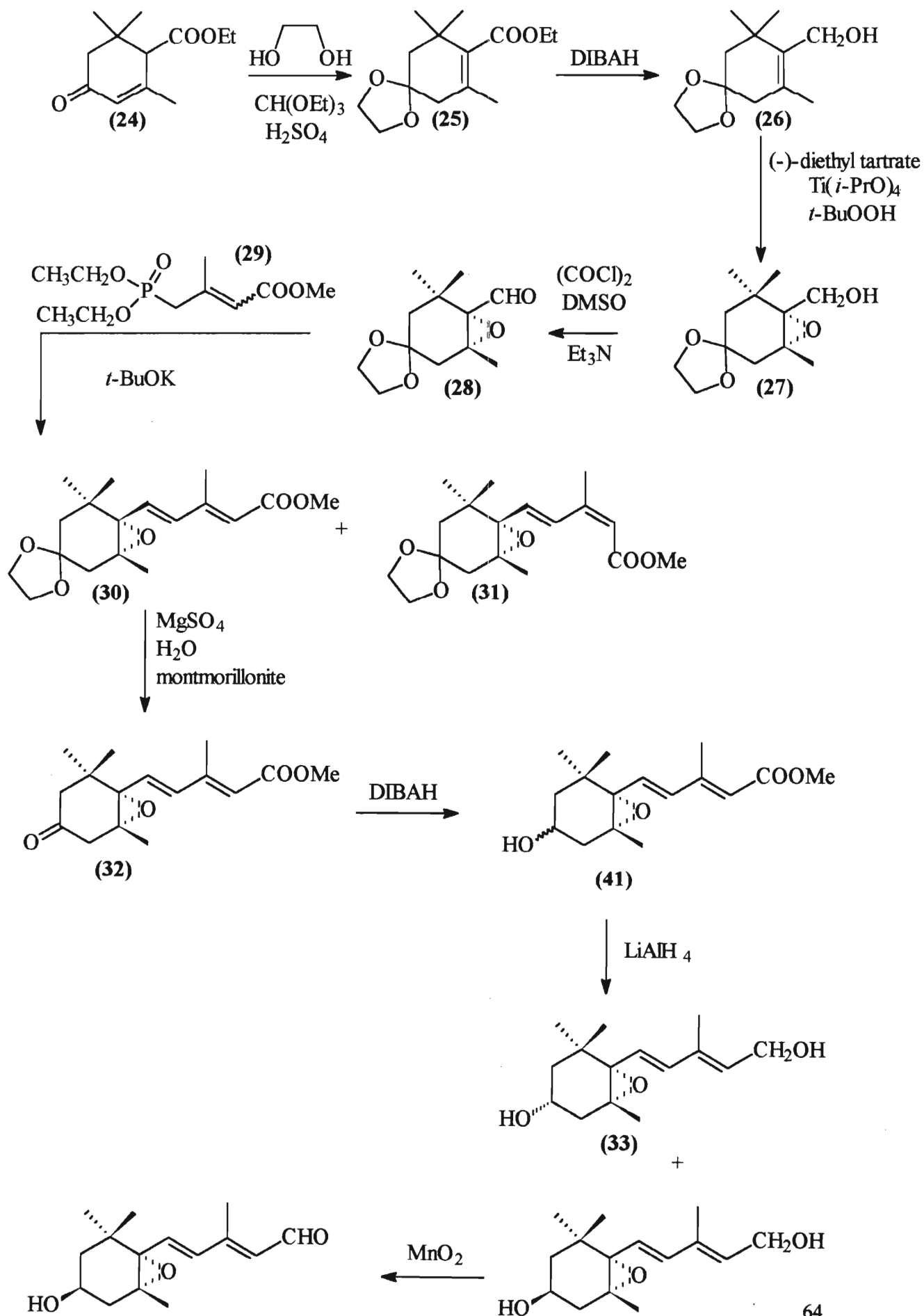
### 3. Experimental

#### 3.1 Index

<u>Compound name</u>	<u>Number</u>	<u>Page</u>
1. Ethyl 2,6,6-trimethyl-4-oxo-2-cyclohexene 1-carboxylate	(24)	67
2. Ethyl 4,6,6-trimethyl-2-oxo-3-cyclohexene 1-carboxylate	(42)	69
3. Ethyl 4,4-ethylenedioxy-2,6,6-trimethyl-1-cyclohexene 1-carboxylate	(25)	69
4. 4,4-Ethylenedioxy-2,6,6-trimethyl-1- cyclohexenemethanol	(26)	70
5. (+)-(1R,2R)-1,2-Epoxy-4,4-(ethylenedioxy)-2,6,6- trimethylcyclohexane-1-methanol	(27)	71
6. 4,4-(Ethylenedioxy)-2,6,6-trimethyl-1,2- epoxycyclohexanemethanol	(28)	73
7. Methyl 3,3- dimethylacrylate	(46)	74
8. Methyl-4-bromosenecioate	(35)	75
9. Diethyl 3-methoxycarbonyl-2-methylprop-2-enyl phosphonate	(29)	76

10. Methyl (1'S,2'R,2E,4E)-5-[4,4-(ethylenedioxy)-2,6,6-trimethyl-1,2-epoxycyclohexyl]-3-methylpenta-2,4-dienoic acid	(30)	78
11. Methyl (1'S,2'R,2Z,4E)-5-[4,4-(ethylenedioxy)-2,6,6-trimethyl-1,2-epoxycyclohexyl]-3-methylpenta-2,4-dienoic acid	(31)	78
12. Methyl(1'S,2'R,2E,4E)-5-(-2,6,6-trimethyl-4-oxo-1,2-epoxycyclohexyl)-3-methylpenta-2,4-dienoic acid	(32)	80
13. Methyl(1'S,2'R,4'S,2E,4E)-5-(4-hydroxy-2,6,6-trimethyl-1,2-epoxycyclohexyl)-3-methylpenta-2,4-dienoic acid	(41)	81
14. (1'S,2'R,4'S,2E,4E)-5-(4-Hydroxy-2,6,6-trimethyl-1,2-epoxycyclohexyl)-3-methylpenta-2,4-dienol	(34)	82
15. (1'S,2'R,4'R,2E,4E)-5-(4-Hydroxy-2,6,6-trimethyl-1,2-epoxycyclohexyl)-3-methylpenta-2,4-dienol	(33)	82
16. (1'S,2'R,4'S,2E,4E)-5-(4-Hydroxy-2,6,6-trimethyl-1,2-epoxycyclohexyl)-3-methyl-penta-2,4-dienal ( <i>trans</i> -Xanthoxin)	(20)	83

**Scheme 6** from p.37



## **3.2 Instrumentation and Chemicals**

### **3.2.1 Purification of solvents and reagents**

All reagents and solvents were dried using standard techniques and distilled prior to use. All unreacted reagents were disposed of carefully<sup>145</sup>. THF was distilled twice over sodium/potassium alloy and benzophenone. All glassware used in reactions carried out under a nitrogen atmosphere was flame dried prior to use.  $\text{ZnCl}_2$  was fused before use. Molecular sieves were dried in a furnace and pulverised prior to use.

Low temperatures were maintained using dry ice/solvent baths according to the procedure of Phipps and Hume.<sup>146</sup>

### **3.2.2 Chromatographic separations**

Kieselgel 60 F<sub>254</sub> Merck aluminium backed plates precoated with 0.25 mm silica gel 60 were used for thin layer chromatography (tlc). Preparative column chromatography was performed using the techniques of Still *et al.*<sup>147</sup> on Merck silica gel (230 – 400 mesh). Centrifugal chromatography was performed on a Harrison Research Chromatotron. Ultraviolet light and anisaldehyde stain (which consists of anisaldehyde and sulphuric acid in an ethanolic solution) were used to detect components following tlc.

### **3.2.3 Spectroscopic and physical data**

Proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectra and decoupled carbon nuclear magnetic resonance ( $^{13}\text{C}$  NMR) spectra were recorded on a Varian Gemini 200 instrument at 199.98 MHz and 50.29 MHz respectively. All spectra were recorded in deuterated chloroform ( $\text{CDCl}_3$ ). Chemical shifts were reported on the  $\delta$  scale relative

to tetramethylsilane as an internal standard. The chemical shifts were reported: value [number of hydrogens, description of signal, assignment, coupling constant(s) in Hz where applicable]. The DEPT pulse sequence was routinely used for the complete assignment of NMR signals.

Gas chromatographic mass spectra were recorded on a Hewlett-Packard gas chromatographic mass spectrometer (HP5988A). Ratios of compounds were calculated based on comparison of peak areas. Analyses were performed under different operating conditions depending on the molecular mass of the compound in question.

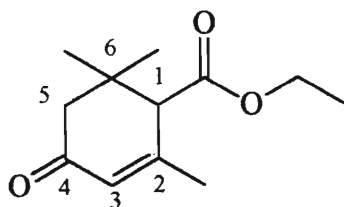
### **3.2.4 Other general procedures**

Concentration or evaporation *in vacuo* refers to the removal of solvent under reduced pressure on a rotary evaporator and final drying on an oil vacuum pump.

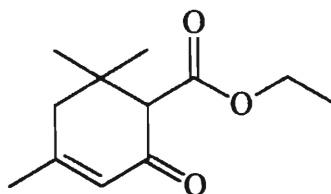
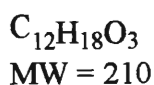
Yields are calculated from the mass of the immediate synthetic precursor used.

### 3.3 Preparations

#### *Ethyl 2,6,6-trimethyl-4-oxo-2-cyclohexene-1-carboxylate (24)*



(24)



(42)

A mixture of redistilled mesityl oxide (58.89 g, 0.60 mol), ethyl acetoacetate (78.09 g, 0.60 mol), anhydrous  $\text{ZnCl}_2$  (12 g, 0.088 mol), heptane (10.3 ml) and benzene (85 ml) was refluxed at  $110^\circ\text{C}$  for 48 hrs.<sup>100,131</sup> The water, which formed during the reaction, was azeotropically distilled and collected in a Dean and Stark trap. The cooled reaction mixture was washed successively with  $\text{H}_2\text{O}$  (150 ml),  $\text{H}_2\text{O}$  (75 ml), 5% aq.  $\text{NaHCO}_3$  (75 ml) and  $\text{H}_2\text{O}$  (75 ml). The organic phase was dried over  $\text{MgSO}_4$  and the solvent removed *in vacuo* yielding a crude oil (79,80 g). The oil was distilled using a fractionating column with efficiency of approximately 10 theoretical plates. Distillation gave isophorone at  $30\text{--}50^\circ\text{C}$ , 0.5 mm Hg (21.88 g) and a condensation product (a yellow oil) at  $112\text{--}121^\circ\text{C}$ , 0.18 mm Hg (126.48g, 34%). The distillate contained two compounds, *ethyl 2,6,6-trimethyl-4-oxo-2-cyclohexene-1-carboxylate (24) ester* and *ethyl 4,6,6-trimethyl-2-oxo-3-cyclohexene-1-carboxylate (42)* in a ratio 4:1 as determined by GC-MS. Analytical samples of **24** and **42** were prepared by column chromatography on silica gel using a solvent system consisting of hexane-ethyl acetate in a ratio 7:3.

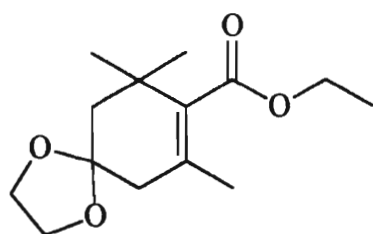
*Ethyl 2,6,6-trimethyl-4-oxo-2-cyclohexene-1-carboxylate (24)*

$^1\text{H}$   $\delta$  1.09 (6H, s,  $2\text{CH}_3$ ), 1.30 (3H, t,  $J = 7.1$ ,  $\text{CH}_2\text{CH}_3$ ), 1.94 (3H, s,  $\text{CH}_3\text{CH}_2$ ), 2.07, 2.78 (2H, AB,  $J = 16.5$ ,  $\text{CH}_2$ ), 2.97 (1H, s,  $\text{CHCOO}$ ), 4.21 (2H, q,  $\text{OCH}_2\text{CH}_3$ ), 5.98 (1H, s,  $\text{CH}=\text{C}$ );  $^{13}\text{C}$   $\delta$  14.3 ( $\text{OCH}_2\text{CH}_3$ ), 23.5 ( $\text{CH}=\text{CCH}_3$ ), 27.2, 28.3 (6H,  $2\text{CH}_3$ ), 35.8 ( $\text{CH}_3\text{CCH}_3$ ), 47.1 ( $\text{C}(\text{CH}_3)_2$ ), 58.4 ( $\text{CCHCOO}$ ), 61.2 ( $\text{COOCH}_2\text{CH}_3$ ), 127.4 ( $\text{COCH}=\text{C}$ ), 154.9 ( $\text{C}=\text{CCH}_3$ ), 170.7 ( $\text{COOCH}_2$ ), 199.0 ( $\text{CH}_2\text{COCH}$ );  $m/z$  (EI) 210 ( $\text{M}^+$ , 16%), 195 (12), 165 (4), 154 (100), 137 (27), 126 (86), 109(46).

*Ethyl 4,6,6-trimethyl-2-oxo-3-cyclohexene-1-carboxylate (42)*

$^1\text{H}$   $\delta$  1.10 (6H, q,  $J = 16.4$ ,  $2\text{CH}_3$ ), 1.26 (3H, t,  $J = 7.1$ ,  $\text{CH}_2\text{CH}_3$ ), 1.98 (3H, s,  $\text{C}=\text{CHCH}_3$ ), 2.09, 2.49 (2H, AB,  $J = 18.6$ ,  $\text{CH}_2$ ), 3.13 (1H, s,  $\text{CHCOOCH}_2$ ), 4.17 (2H, q,  $J = 7.1$ ,  $\text{OCH}_2\text{CH}_3$ ), 5.93 (1H, s,  $\text{CH}=\text{C}$ );  $^{13}\text{C}$   $\delta$  14.2 ( $\text{OCH}_2\text{CH}_3$ ), 24.8 ( $\text{CH}=\text{CCH}_3$ ), 25.3, 28.3 ( $2\text{CH}_3$ ), 36.0 ( $\text{CH}_3\text{CCH}_3$ ), 44.1 ( $\text{C}=\text{CCH}_2$ ), 60.8 ( $\text{OCH}_2\text{CH}_3$ ), 63.5 ( $\text{CCHCOOCH}_2$ ), 124.4 ( $\text{COCH}=\text{CCH}_3$ ), 161.4 ( $\text{CH}_3\text{C}=\text{CH}$ ), 169.0 ( $\text{COOCH}_3$ ), 194.4 ( $\text{CHCOCH}$ );  $m/z$  (EI) 210 ( $\text{M}^+$ , 59%), 196 (17), 165 (31), 149 (100), 137 (33), 123 (61), 108 (19).

Ethyl 4,4-ethylenedioxy-2,6,6-trimethyl-1-cyclohexene-1-carboxylate (**25**) from (**24**)



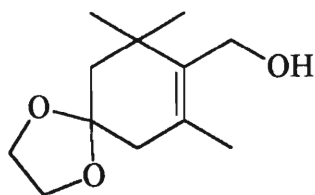
$C_{14}H_{22}O_4$   
MW = 254

(**25**)

A solution of a mixture **24** and **42** in the ratio 4:1 (40 g, 0.19 mol), ethane-1,2-diol (78.85 ml) and triethyl orthoformate (88.17 ml) were mixed under  $N_2$  at  $12^\circ C$ , conc  $H_2SO_4$  (0.17 ml) was added dropwise and the mixture was stirred at the same temperature for 40 min (the solution turned blue).<sup>100</sup> The mixture was then stirred at r.t. in the dark for 20 hrs. It was then diluted with hexane (263 ml), washed twice with sat. aq.  $NaHCO_3$  soln. (65.7 ml) and twice with  $H_2O$  (65.7 ml) The combined aqueous phases were extracted with hexane (78.9 ml). The organic phases were combined, dried over  $MgSO_4$ , filtered and the solvent was removed *in vacuo* to obtain a crude oil (58.59 g). The oil was distilled using a fractionating column of approximately 10 theoretical plates collecting *Fr.1*,  $72-104^\circ C$ , 0.30 mm Hg, 19.50g (**25** with 50% of **42**), *Fr.2*,  $104-130^\circ C$ , 0.29 mm Hg, 18.22g (**13**). Redistillation of *Fr.1* gave 10.66 g (**25**). Combination of fractions gave **25** (28.79 g, 60%).

$^1H$   $\delta$  1.19 (6H, s,  $2CH_3$ ), 1.32 (3H, t,  $J = 7.1$ ,  $CH_3CH_2O$ ), 1.70 (5H, s,  $CH_2C(CH_3)_2$ ,  $CH_3C=$ ), 2.29 (2H, s,  $CH_2C=$ ), 3.96 (4H, s,  $OCH_2CH_2O$ ), 4.24 (2H, q,  $J = 7.1$ ,  $CH_3CH_2O$ );  $^{13}C$   $\delta$  14.4 ( $OCH_2CH_3$ ), 21.1 ( $2CH_3C$ ), 28.9 ( $CH_3C$ ), 36.0 ( $CH_3CCH_3$ ), 41.4 ( $((CH_3)_2CH_2C(OCH_2)_2$ ), 45.1 ( $((OCH_2)_2CH_2C=$ ), 60.2 ( $CH_3CH_2O$ ), 64.1 ( $OCH_2CH_2O$ ), 107.5 ( $((OCH_2)_2C$ ), 130.4 ( $C=CCOOCH_2$ ), 134.4 ( $C=CCOOCH_2$ ), 170.3 ( $CCOOCH_2$ );  $m/z$  (EI) 254 ( $M^+$ , 12%), 209 (14), 193 (3) 168 (2), 139 (4) 123 (15), 86 (100), 67 (3).

4,4-Ethylenedioxy-2,6,6-trimethyl-1-cyclohexenemethanol (**26**) from (**25**)



$C_{12}H_{20}O_3$   
MW = 212

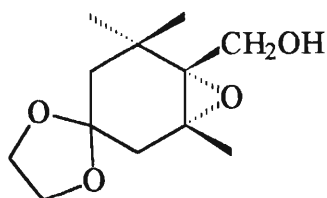
(**26**)

A solution of ethyl 4,4-ethylenedioxy-2,6,6-trimethyl-1-cyclohexene-1-carboxylate (19 g, 0.075 mol) in toluene (60 ml) under  $N_2$  was cooled to  $-40^\circ C$  and DIBAH in hexane solution (1 M) (187.5 ml, 2.50 equiv.) was added.<sup>132</sup> The reaction was left to stir overnight before quenching with a small amount of methanol and sat. aq.  $NH_4Cl$  soln. The mixture was extracted with ether, the organic layer was washed with sat. aq. NaCl solution dried over  $MgSO_4$  and the solvent removed *in vacuo* to afford **26** as a yellow oil (11.86 g, 74%).

$^1H$   $\delta$  1.14 (6H, s,  $2CH_3C$ ), 1.69 (2H, s,  $CH_2C(CH_3)_2$ ), 1.80 (3H, s,  $CH_3C=$ ), 2.29 (2H, s,  $CH_2C=$ ), 3.96 (4H, s,  $OCH_2CH_2O$ ), 4.17 (2H, s,  $CH_2OH$ );  $^{13}C$   $\delta$  19.6 ( $CH_3C$ ), 28.9 ( $2CH_3C$ ), 37.0 ( $((CH_3)_2CC=$ ), 42.4 ( $CH_2C(CH_3)_2$ ), 45.4 ( $CH_2C=$ ), 58.3 ( $CH_2OH$ ), 64.0 ( $OCH_2CH_2O$ ), 107.5 ( $CH_2OCOCH_2$ ), 130.3 ( $CH_3C=$ ), 136.9 ( $HOCH_2C=$ ),  $m/z$  (EI) 212 ( $M^+$ , 7%), 179 (2), 169 (1), 111 (6), 86 (100), 55(16).

(+)-(1*R*,2*R*)-1,2-Epoxy-4,4-(ethylenedioxy)-2,6,6-trimethylcyclohexan-1-methanol

(27) from (26)



$C_{12}H_{20}O_4$   
MW = 228

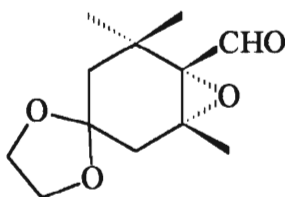
(27)

A stirred mixture of 4Å Molecular sieves (freshly activated and pulverised) (1.40 g) in  $CH_2Cl_2$ , diethyl D-(-)-tartrate (1.91 g, 9.30 mmol) and titanium (IV) isopropoxide (1.87 g, 6.60 mmol), under  $N_2$  was cooled to  $-40^\circ C$  and treated with 5-6M *t*-BuOOH<sup>100,134</sup> solution in decane (precooled to  $4^\circ C$ ) (10.9 ml, 0.06 mol) and diluted to 20.5 ml with  $CH_2Cl_2$ . The reaction mixture was then cooled to  $-60^\circ C$  and after 45 min the alcohol **26** (7.0 g, 0.033 mol) in 7 ml  $CH_2Cl_2$  was added slowly over 65 minutes. The reaction mixture was then warmed up to  $-20^\circ C$  and stirred overnight. The reaction was quenched with  $H_2O$  (20 ml) and the resulting mixture stirred for 30 min at room temperature. Then a 30% NaOH solution in brine (3 ml) was added and the solution was stirred for a further 30 min. The mixture was poured into a separating funnel and the  $CH_2Cl_2$  phase was run off. The aqueous phase was stirred with  $CH_2Cl_2$  (20 ml) and filtered through celite. The combined  $CH_2Cl_2$  phases were dried over  $MgSO_4$  and the solvent was removed *in vacuo*. The unreacted *t*BuOOH was distilled off at room temperature at 0.5 mm Hg yielding a pure product **27** (7.48g, 99%).

$^1H$   $\delta$  1.09 (3H, s,  $\underline{CH_3}$ ), 1.18 (3H, s,  $\underline{CH_3}$ ), 1.31 (1H, dd,  $^2J=13.7$ ,  $^4J=2.1$ ,  $\underline{H_{eq}CCH_3}$ ), 1.41 (3H, s,  $\underline{CH_3}$ ), 1.62 (1H, d,  $J=13.7$ ,  $\underline{H_{ax}CCH_3}$ ), 1.99 (1H, dd,  $^2J=15.6$ ,  $^4J=1.9$ ,  $\underline{H_{eq}COCH_2CH_2O}$ ), 2.24 (1H, d,  $J=15.6$ ,  $\underline{H_{ax}COCH_2CH_2O}$ ), 3.67-3.95 (6H, m,  $\underline{CH_2OH}$ ,  $\underline{OCH_2CH_2O}$ );  $^{13}C$   $\delta$  21.0 ( $\underline{CH_3COC}$ ), 24.3 ( $\underline{CH_3CCCH_2OH}$ ), 26.5 ( $\underline{CH_3CCCH_2OH}$ ), 34.5 ( $\underline{CH_3CCH_3}$ ), 41.6 ( $\underline{CCH_2CCH_3}$ ), 42.6 ( $\underline{CCH_2C(CH_3)_2}$ ), 58.3 ( $\underline{CCH_2OH}$ ), 63.6 ( $\underline{CH_2OC}$ ), 64.0 ( $\underline{CH_2OC}$ ), 64.1 ( $\underline{CH_3COC}$ ), 68.46

(CH<sub>3</sub>COCH<sub>2</sub>OH), 106.8 (CH<sub>2</sub>OCOCH<sub>2</sub>); *m/z* (EI) 213 (M<sup>+</sup> - CH<sub>3</sub>, 3%), 197 (27), 171 (14), 142 (50), 129 (47), 112 (42), 84 (100), 55(21); [α]<sub>D</sub> = + 19.14° (lit.<sup>99</sup> = +22.9°).

4,4-(Ethylenedioxy)-2,6,6-trimethyl-1,2-epoxycyclohexane-1-carboxaldehyde (28)  
from (27)



$C_{12}H_{18}O_4$   
MW = 226

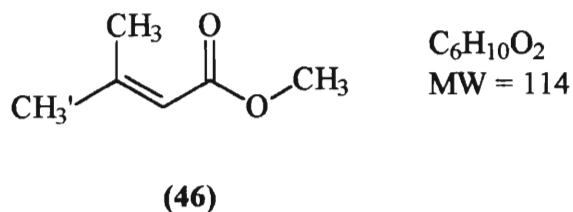
(28)

Oxalyl chloride (0.2 ml, 2.4 mmol) and dimethyl sulfoxide (0.4 ml, 4.3 mmol) were mixed in  $CH_2Cl_2$  (5.5 ml) under  $N_2$  at  $-50^\circ C$ .<sup>136</sup> After 2 min **27** (0.50g, 2.2 mmol) in  $CH_2Cl_2$  (2.4 ml) was added to the mixture within 5 minutes. After 20 min triethylamine (2.3 ml, 0.02 mol) was added and the mixture was stirred at the same temperature for 10 min. The mixture was then allowed to warm up to r.t. Water was then added and the mixture was extracted with  $CH_2Cl_2$ . The organic layer was washed with  $H_2O$ , dried over  $Na_2SO_4$  and the solvent removed *in vacuo* to yield crude product **28** (0.450 g, 90%).

$^1H$   $\delta$  1.08 (3H, s,  $CH_3C(6)$ ), 1.32 (3H, s,  $CH_3C(6)$ ), 1.32 (1H, dd,  $^2J=13.7$ ,  $^4J=2.0$ ,  $H_{eq}C(5)$ ), 1.47 (3H, s,  $CH_3C(2)$ ), 1.71 (1H, d,  $J=13.9$ ,  $H_{ax}C(5)$ ), 2.08 (1H, dd,  $^2J=15.8$ ,  $^4J=1.6$ ,  $H_{eq}C(3)$ ), 2.30 (1H, d,  $J=15.8$ ,  $H_{ax}C(3)$ ), 3.90 (4H, m,  $OCH_2CH_2O$ ), 9.79 (1H, s,  $CHO$ );  $^{13}C$   $\delta$  20.9 ( $CH_3C(2)$ ), 24.8 ( $CH_3C(6)$ ), 27.0 ( $CH_3C(6)$ ), 33.9 ( $CH_3C(6)$ ), 41.2 ( $CH_2(3)$ ), 42.9 ( $CH_2(5)$ ), 63.7 ( $CH_2O$ ), 64.2 ( $CH_2O$ ), 64.4 ( $C(2)$ ), 72.5 ( $C(1)$ ), 106.6 ( $OCH_2CCH_2O$ ), 200.8 ( $O=CH$ );  $m/z$  (EI) 226 ( $M^+$ , 1%), 197 (49), 169 (6), 141 (17), 127 (46), 111 (38), 97 (5), 86 (100), 43 (14);  $[\alpha]_D = -24.69$

(lit.<sup>99</sup> = -57).

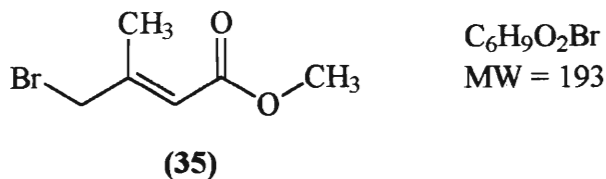
*Methyl 3,3- dimethylacrylate (46)*



To a stirred solution of methanol (200 ml) and Methyl 3,3- dimethylacrylic acid (20 g, 0.20 mol), conc HCl (7.98 ml) was added dropwise.<sup>137,138</sup> The mixture was heated under reflux for 24 hrs, cooled, poured onto water and the ester was extracted with diethyl ether. The ethereal solution was washed with water, then with sat. NaHCO<sub>3</sub> and dried over anhydrous MgSO<sub>4</sub>. The ether was removed *in vacuo* to yield methyl 3,3- dimethylacrylate (46) a yellow liquid (13.19g, 58%).

<sup>1</sup>H δ 1.09 (3H, s, CH<sub>3</sub>), 2.18 (3H, s, CH<sub>3</sub>'), 3.68 (3H, s, OCH<sub>3</sub>), 5.69 (1H, s, CHCOOCH<sub>3</sub>); <sup>13</sup>C δ 20.2 (CH<sub>3</sub>), 27.4 (CH<sub>3</sub>'), 50.8 (OCH<sub>3</sub>), 115.7 (C=CH), 156.8 (CCHCO), 167.2 (CO); *m/z* (EI) 114 (M<sup>+</sup>, 50%), 99 (7), 83 (100), 67 (3), 55 (43).

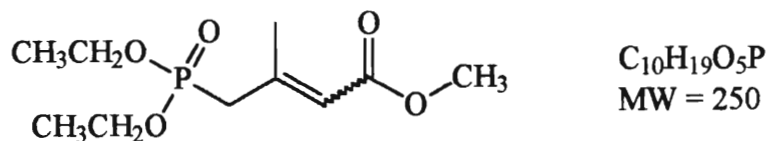
Methyl 4-bromosenecioate (**35**) from (**46**)



A mixture of **46** (3.79 g, 33.2 mmol), benzoyl peroxide (0.33 g, 1.4 mmol), quinol and NBS (0.5 g, 2.81 mmol) was heated to reflux (136°C).<sup>148,139</sup> NBS (5.43g, 30.5 mmol) was added portionwise over 10 min. The mixture was refluxed for a further 5 min, cooled and diluted with CCl<sub>4</sub>. The precipitate was filtered with suction and the solvent was removed from the filtrate *in vacuo*. Distillation of the mixture gave the bromide **35** (2.53g, 39%), b.p.30-35°C /0.12 torr. (lit.<sup>137</sup> 84-85°C/12 torr).

<sup>1</sup>H δ 2.28 (3H, s, CH<sub>3</sub>C=C), 3.719 (3H, s, OCH<sub>3</sub>), 3.95 (2H, s, CH<sub>2</sub>Br), 5.97 (1H, s, C=CH); <sup>13</sup>C δ 17.2 (CH<sub>3</sub>), 38.2 (CH<sub>2</sub>), 51.23 (OCH<sub>3</sub>), 119.0 (CH), 152.8 (CCH<sub>3</sub>), 166.2 (COO); *m/z* (EI) 193 (M<sup>+</sup>, 40%), 191 (44), 161 (41), 148 (1), 133 (11), 113 (74), 82 (82), 67 (12), 53 (100).

Diethyl 3-methoxycarbonyl-2-methylprop-2-enyl phosphonate (**29**) from (**35**)



(**29**)

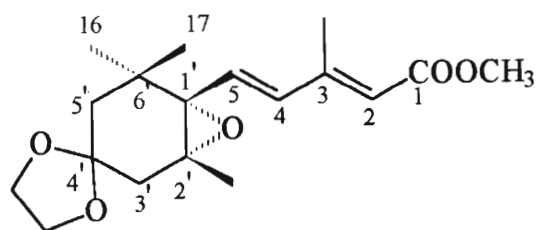
Methyl 4-bromosenecioate (**35**) (9.07 g, 0.05 mol) was slowly added to triethyl phosphite (10.88 g, 0.06 mol) at 100°C.<sup>141</sup> The temperature was slowly raised to 120°C and maintained at that temperature for 1 hour. Distillation of the mixture gave the *phosphonate* (8.73 g, 74%), b.p. 110°C/0.5 torr. GC-MS the showed the presence of two isomers in the ratio 3:1. Looking at the <sup>1</sup>H NMR spectrum of **17** the signal at 2.32 ppm clearly represents 3 protons while the signal further upfield at 2.07 ppm is a similar yet too small for 3 protons signal. The signal at 2.70 ppm with a J=23.4 resrepresents  $\underline{CH}_2C=$  while the signal at 3.48 ppm with J=27.4 is small for 2 protons. Since the coupling constants of *Z* phosphonates is expected to be larger than the *E* phosphanates the larger signals would resrepresent the *E* isomer. Comparing the integrals of the two signals the *E/Z* ratio was determined.

	<i>E</i>	<i>Z</i>
NMR	3	1
GC-MS	2.9	1

<sup>1</sup>H δ 1.33 (6H, m, 2(OCH<sub>2</sub>CH<sub>3</sub>)), 2.07 (3H, m,  $\underline{CH}_3C=C$  {*Z*}), 2.32 (3H, m,  $\underline{CH}_3C=C$  {*E*}), 2.70 (2H, d, J = 23.4,  $\underline{CH}_2C=C$  {*E*}), 3.48 (2H, d, J = 27.4,  $\underline{CH}_2C=C$  {*Z*}), 3.69 (3H, s, OCH<sub>3</sub>), 4.12 (4H, m, 2(OCH<sub>2</sub>CH<sub>3</sub>)), 5.82 (H, s,  $\underline{CH}=C$ ); <sup>13</sup>C δ 16.4

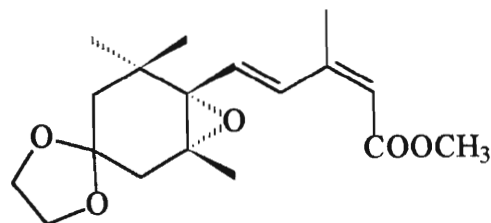
(2(OCH<sub>2</sub>CH<sub>3</sub>), 20.1 (CH<sub>3</sub>C=C {E}), 26.2 (CH<sub>3</sub>C=C {Z}), 30.0, 32.6 (CH<sub>2</sub>PO {Z}), 37.2 39.9 (CH<sub>2</sub>PO {E}), 51.0 (OCH<sub>3</sub>), 62.3 (2(OCH<sub>2</sub>CH<sub>3</sub>)), 118.4, 118.6 (CH<sub>3</sub>C=CH {Z}), 119.42, 119.6 (CH<sub>3</sub>C=CH {E}), 150.0 (CH<sub>3</sub>C=CH), 166.4 (C=O), *m/z* (EI) 250 (M<sup>+</sup>, 4%), 218 (29), 190 (34), 162 (53), 134 (100), 109 (15), 82 (33), 53 (30).

Methyl (1*S*,2*R*,2*E*,4*E*)-5-[4,4-(ethylenedioxy)-2,6,6-trimethyl-1,2-epoxycyclohexyl]-3-methylpenta-2,4-dienoic acid (**30**) and (**31**) from (**29**) and (**28**)



(**30**)

$C_{18}H_{26}O_5$   
MW = 322



(**31**)

To a mixture of *t*-BuOK (0.173 g, 1.36 mmol) in THF (3.47 ml) under  $N_2$  at  $0^\circ C$ , diethyl 3-methoxycarbonyl-2-methylprop-2-enyl phosphate (*E* : *Z* = 3 : 1) (**29**) (0.482 g, 1.9 mmol) in THF (1.35 ml) was added.<sup>100</sup> After 30 min, the aldehyde (**28**) (0.193 g, 0.8 mmol) in THF (0.77 ml) was added slowly and the resulting mixture stirred for 4 hrs at r.t.. The mixture was treated with water, then sat. NaCl and extracted with diethyl ether. The organic layer was washed with aq. NaCl, then  $H_2O$  and dried over  $Na_2SO_4$ . The solvent was then removed *in vacuo* yielding a mixture of **30** and **31** (0.352 g). The mixture was separated by means of a chromatotron using diethyl ether/hexane:1:10 + 0.5% methanol. The separation yielded **30** (0.128 g) and **31** (0.020 g) [total 0.148 g, 54 %].

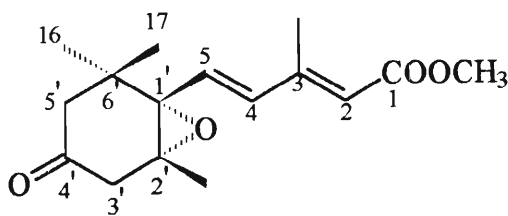
$^1H$   $\delta$  0.96 (3H, s,  $CH_3(16)$ ), 1.17 (3H, s,  $CH_3(17)$ ), 1.26 (3H, s,  $CH_3C(2')$ ), 1.35 (1H, dd,  $^2J = 13.9$ ,  $^4J = 2.0$ ,  $H_{eq}C(5')$ ), 1.71 (1H, d,  $J = 13.7$ ,  $H_{ax}C(5')$ ), 2.05 (1H, dd,  $^2J = 15.8$ ,  $^4J = 1.9$ ,  $H_{eq}C(3')$ ), 2.27 (1H, d,  $J = 15.7$ ,  $H_{ax}C(3')$ ), 2.30 (3H, d,  $J = 1.2$ ,  $CH_3C(3)$ ), 3.71 (3H, m,  $OCH_3$ ), 3.84 (4H, m,  $OCH_2CCH_2O$ ), 5.81 (1H, m,  $HC(2)$ ), 6.33 (2H, d,  $J = 1.1$ ,  $HC(5)$ ,  $HC(4)$ );  $^{13}C$   $\delta$  13.9 ( $CH_3(3)$ ), 21.1 ( $CH_3(2')$ ), 25.9 ( $CH_3(6')$ ), 27.4 ( $CH_3(6')$ ), 35.2 ( $C(6')$ ), 41.7 ( $CH_2(3')$ ), 42.3 ( $CH_2(5')$ ), 51.0 ( $OCH_3$ ), 62.6 ( $OCH_2$ ), 64.1 ( $OCH_2$ ), 64.9 ( $C(2')$ ), 70.8 ( $C(1')$ ), 107.0 ( $C(4')$ ), 119.30 ( $CH(2)$ ),

130.6 ( $\underline{\text{CH}}(5)$ ), 136.8 ( $\underline{\text{CH}}(4)$ ), 151.1 ( $\underline{\text{C}}(3)$ ), 167.5 ( $\underline{\text{COOCH}}_3$ );  $m/z$  (EI) 322 ( $\text{M}^+$ , 41%), 263 (2), 236 (73), 221 (100), 177 (31), 161 (28), 133 (45), 127 (9), 119 (43), 113 (12), 87 (29);  $[\alpha]_{\text{D}} = -13.30$  (lit.<sup>99</sup> - 43.7)

*Methyl (1S,2R,2Z,4E)-5-[4,4-(ethylenedioxy)-2,6,6-trimethyl-1,2-epoxycyclohexyl]-3-methylpenta-2,4-dieneoic acid (31)*

$^1\text{H}$   $\delta$  0.99 (3H, s,  $\underline{\text{CH}}_3(16)$ ), 1.22 (3H, s,  $\underline{\text{CH}}_3(17)$ ), 1.25 (3H, s,  $\underline{\text{CH}}_3\text{C}(2')$ ), 1.34 (1H, dd,  $^2J = 13.7$ ,  $^4J = 2.1$ ,  $\underline{\text{H}}_{\text{eq}}\text{C}(5')$ ), 1.74 (1H, d,  $J = 13.6$ ,  $\underline{\text{H}}_{\text{ax}}\text{C}(5')$ ), 2.0 (3H, d,  $J = 1.3$ ,  $\underline{\text{CH}}_3\text{C}(3)$ ), 2.04 (1H, dd,  $^2J = 15.7$ ,  $^4J = 2.0$ ,  $\underline{\text{H}}_{\text{eq}}\text{C}(3')$ ), 2.28 (1H, d,  $J = 15.7$ ,  $\underline{\text{H}}_{\text{ax}}\text{C}(3')$ ), 3.70 (3H, s,  $\text{OCH}_3$ ), 3.89 (4H, m,  $\text{OCH}_2\text{CCH}_2\text{O}$ ), 5.70 (1H, m,  $\underline{\text{HC}}(2)$ ), 6.29 (1H, AB,  $J_{\text{AB}} = 16.0$ ,  $\underline{\text{HC}}(4)$ ), 7.63 (1H, AB,  $J_{\text{AB}} = 16.0$ ,  $\underline{\text{HC}}(5)$ );  $^{13}\text{C}$   $\delta$  21.1 ( $\underline{\text{CH}}_3\text{C}(2')$ ), 21.4 ( $\underline{\text{CH}}_3\text{C}(3)$ ), 25.9 ( $\underline{\text{CH}}_3\text{C}(6')$ ), 27.5 ( $\underline{\text{CH}}_3\text{C}(6')$ ), 35.1 ( $\underline{\text{C}}(6')$ ), 41.1 ( $\underline{\text{CH}}_2(3')$ ), 42.3 ( $\underline{\text{CH}}_2(5')$ ), 51.1 ( $\text{OCH}_3$ ), 63.6 ( $\text{OCH}_2$ ), 64.1 ( $\text{OCH}_2$ ), 64.7 ( $\underline{\text{C}}(2')$ ), 70.6 ( $\underline{\text{C}}(1')$ ), 107.1 ( $\underline{\text{C}}(4')$ ), 117.5 ( $\underline{\text{CH}}(2)$ ), 131.2 ( $\underline{\text{CH}}(4)$ ), 131.8 ( $\underline{\text{CH}}(5)$ ), 149.3 ( $\underline{\text{C}}(3)$ ), 166.4 ( $\underline{\text{COOCH}}_3$ );  $m/z$  (EI) 322 ( $\text{M}^+$ , 25%), 263 (1), 251 (1.5), 236 (34), 221 (100), 204 (16), 193 (15), 189 (12), 177 (36), 133 (54), 123 (40), 119 (58), 87 (43).

Methyl(1*S*,2*R*,2*E*,4*E*)-5-(-2,6,6-trimethyl-4-oxo-1,2-epoxycyclohexyl)-3-methylpenta-2,4-dienoic acid (**32**) from (**30**)



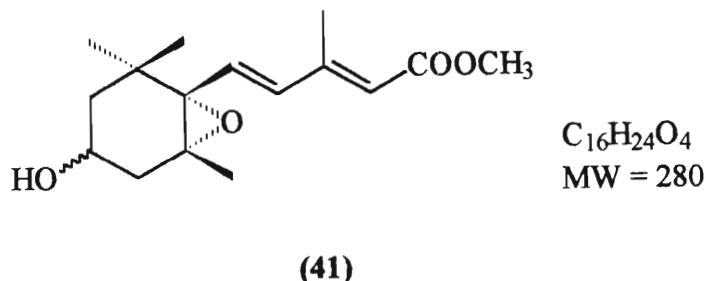
C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>  
MW = 278

(**32**)

A mixture of **30** (0.122 g, 0.378 mmol) in toluene (4.88 ml) was treated with Montmorillonite clay (0.976 g), MgSO<sub>4</sub> (0.61 g) and H<sub>2</sub>O (0.061 ml).<sup>100</sup> The flask was stoppered, shaken vigorously for 2.5 min and EtOAc (4.88 ml) was added. The mixture was filtered through a layer of celite and the solvents removed *in vacuo* yielding a yellow oil **32** (0.0856 g, 81%).

<sup>1</sup>H δ 1.05 (3H, s, CH<sub>3</sub>(16)), 1.16 (3H, s, CH<sub>3</sub>(17)), 1.23 (3H, s, CH<sub>3</sub>C(2')), 1.98, 2.59 (2H, *J*<sub>AB</sub> = 15.4, H<sub>2</sub>C(5')), 2.33 (3H, d, *J* = 1.1, CH<sub>3</sub>C(3)), 2.59, 2.88 (2H, *J*<sub>AB</sub> = 19.9, H<sub>2</sub>C(3')), 3.73 (3H, m, OCH<sub>3</sub>), 5.85 (1H, m, HC(2)), 6.39 (2H, d, *J* = 4.2, HC(5), HC(4)); <sup>13</sup>C δ 13.9 (CH<sub>3</sub>(3)), 19.4 (CH<sub>3</sub>(2')), 24.9 (CH<sub>3</sub>(6')), 26.7 (CH<sub>3</sub>(6')), 36.0 (C(6')), 43.6 (CH<sub>2</sub>(3')), 50.9 (CH<sub>2</sub>(5')), 51.2 (OCH<sub>3</sub>), 63.3 (C(2')), 70.5 (C(1')), 120.0 (CH(2)), 128.2 (CH(4)), 137.2 (CH(5)), 150.5 (C(3)), 167.3 (COOCH<sub>3</sub>), 207.2 (C=O); *m/z* (EI) 278 (M<sup>+</sup>, 0.6 %), 260 (2), 247 (2), 231 (0.5), 222 (10), 190 (100), 162 (32), 147 (12), 134 (35), 105 (15), 91 (48), 65 (19).

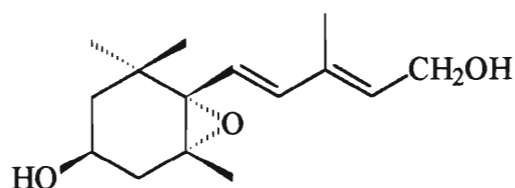
Methyl(1*S*,2*R*,4*S*,2*E*,4*E*)-5-(4-hydroxy-2,6,6-trimethyl-1,2-epoxycyclohexyl)-3-methylpenta-2,4-dienoic acid (**41**) from (**32**)



A solution of **32** (0.075 g, 0.3 mmol) in toluene (6.5 ml) under N<sub>2</sub> was cooled to -78°C and DIBAH in hexane solution (1M) (2.3 ml, 5.0 equiv.) was added.<sup>101</sup> The reaction mixture was stirred for 24 hrs before quenching with a small amount of methanol and sat. aq. NH<sub>4</sub>Cl solution. The mixture was extracted with diethyl ether, the organic layer washed with sat. aq. NaCl solution, and dried over MgSO<sub>4</sub>. The solvent was removed *in vacuo*. The crude product was purified by means of a chromatotron using hexane/ethyl acetate = 4:1 to afford **41** a yellow oil (0.062 g, 82%).

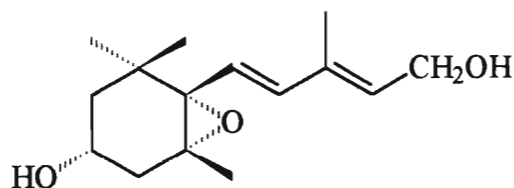
<sup>1</sup>H δ 0.97 (3H, s, CH<sub>3</sub>(16)), 1.16 (3H, s, CH<sub>3</sub>(17)), 1.18 (3H, s, CH<sub>3</sub>C(2')), 1.21 (1H, dd, <sup>2</sup>J = 1.0, <sup>4</sup>J = 0.5, H<sub>ax</sub>C(5')), 1.62 (3H, m, OH, H<sub>eq</sub>C(5'), H<sub>ax</sub>C(3)), 2.29 (3H, s, CH<sub>3</sub>C(3)), 2.40 (1H, ddd, H<sub>eq</sub>C(3')), 3.71 (3H, s, OCH<sub>3</sub>), 3.90 (1H, m, HC(4')), 5.802 (1H, d, J = 1.2, HC(2)), 6.30 (2H, d, J = 2.4, HC(5), HC(4)); <sup>13</sup>C δ 13.9 (CH<sub>3</sub>(3)), 19.9 (CH<sub>3</sub>(2')), 24.8 (CH<sub>3</sub>(6')), 29.4 (CH<sub>3</sub>(6')), 35.2 (C(6')), 40.8 (CH<sub>2</sub>(3')), 46.9 (CH<sub>2</sub>(5')), 51.1 (OCH<sub>3</sub>), 64.0 (CHOH), 67.1 (C(2')), 70.0 (C(1')), 119.3 (CH(2)), 131.6 (CH(4)), 136.2 (CH(5)), 151.1 (C(3)), 167.5 (COOCH<sub>3</sub>); *m/z* (EI) 280 (M<sup>+</sup>, 9.8 %), 248 (13), 221 (19), 179 (29), 147 (50), 161 (23), 123 (100), 109 (21), 91 (45), 77 (32), 59 (24).

(1*S*,2*R*,4*S*,2*E*,4*E*)-5-(4-Hydroxy-2,6,6-trimethyl-1,2-epoxycyclohexyl)-3-methylpenta-2,4-dienol (**34**)



(**34**)

$C_{15}H_{24}O_3$   
MW = 252

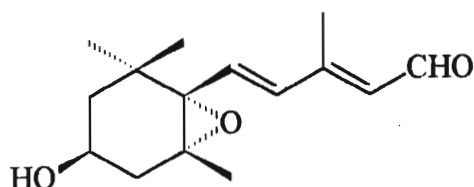


(**33**)

To a suspension of lithium aluminium hydride (0.005 g, 0.1 mmol) in THF (0.8 ml) under  $N_2$  at  $-25^\circ C$ , **41** (0.031 g, 0.1 mmol) in THF (0.2 ml) was added.<sup>132</sup> The mixture was stirred at r.t. for 2 hrs and the reaction quenched with water and 15% aq. sodium hydroxide solution. After stirring for 1 hr the solid suspension was filtered off and washed with THF. The combined filtrate and washings were dried over  $Na_2SO_4$  and the solvent removed *in vacuo* to yield a yellow oil. The crude product was purified by means of a chromatotron using hexane/ $Et_2O$  = 3:2 + 1% MeOH to give a mixture of **22** and **21** (0.011 g, 39%) in a ratio of 15:1 as estimated by GCMS.

$^1H$   $\delta$  0.97 (3H, s,  $\underline{CH_3}$ (16)), 1.14 (3H, s,  $\underline{CH_3}$ (17)), 1.18 (3H, s,  $\underline{CH_3}$ C(2)), 1.27 (1H, dd,  $^2J = 10.7$ ,  $^4J = 2.0$ ,  $\underline{H_{ax}}$ C(5)), 1.62 (1H, dd,  $^2J = 14.2$ ,  $^4J \approx 9$ ,  $\underline{H_{ax}}$ C(3)), 1.63 (1H, ddd,  $^2J = 13$ ,  $^3J = 3.5$ ,  $^4J = 1.9$ ,  $\underline{H_{eq}}$ C(5)), 1.81 (3H, d,  $J = 0.5$ ,  $\underline{CH_3}$ C(9)), 2.37 (1H, ddd,  $^2J = 14.2$ ,  $^3J = 5.0$ ,  $^4J = 1.9$ ,  $\underline{H_{eq}}$ C(3)), 3.90 (1H, m,  $\underline{HC}$ (4)), 4.29 (1H, d,  $J = 6.9$ ,  $\underline{CH_2}$ (11)) 5.69 (1H, t,  $\underline{HC}$ (10)), 5.88, 6.30 (2H, AB,  $J = 15.6$ ,  $\underline{HC}$ (7),  $\underline{HC}$ (8));  $^{13}C$   $\delta$  12.7 ( $\underline{CH_3}$ (9)), 20.0 ( $\underline{CH_3}$ (2)), 24.8 ( $\underline{CH_3}$ (6)), 29.5 ( $\underline{CH_3}$ (6)), 35.2 ( $\underline{C}$ (6)), 40.9 ( $\underline{CH_2}$ (3)), 47.1 ( $\underline{CH_2}$ (5)), 59.3 ( $\underline{CH_2OH}$ ), 64.2 ( $\underline{CHOH}$ ), 66.8 ( $\underline{C}$ (2)), 70.1 ( $\underline{C}$ (1)), 124.5 ( $\underline{CH}$ (7)), 130.2 ( $\underline{CH}$ (10)), 136.6 ( $\underline{CH}$ (8)), 137.4 ( $\underline{C}$ (9));  $m/z$  (EI) 252 ( $M^+$ , 1.3 %), 234 (4), 221 (6), 193 (1.7), 177 (3), 147 (8), 133 (32), 123 (100), 109 (33), 91 (30), 77 (22), 67 (23), 57 (10).

(1*S*,2*R*,4*S*,2*E*,4*E*)-5-(4-Hydroxy-2,6,6-trimethyl-1,2-epoxycyclohexyl)-3-methyl-penta-2,4-dienal (*trans*-Xanthoxin) (**20**) from (**34**)



C<sub>15</sub>H<sub>22</sub>O<sub>3</sub>  
MW = 250

(**20**)

A mixture of **34** (7 mg, 0.03 mmol) and manganese dioxide (60 mg, 28.5 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (1.4 ml) was stirred at room temperature for 2 days.<sup>101</sup>

The mixture was filtered and the solvent removed *in vacuo* yielding an amorphous solid **20** (5 mg, 74%).

<sup>1</sup>H δ 0.98 (3H, s, CH<sub>3</sub>(16)), 1.19 (3H, s, CH<sub>3</sub>(2')), 1.25 (3H, s, CH<sub>3</sub>C(17)), 1.32 (1H, dd, <sup>2</sup>J = 11.5, <sup>3</sup>J = 8.7, H<sub>ax</sub>C(5')), 1.65 (2H, m, OH, H<sub>eq</sub>C(3')), 1.67 (1H, dd, <sup>2</sup>J = 14.4, <sup>3</sup>J = 8.7, <sup>4</sup>J = 1.7, H<sub>ax</sub>C(3')), 2.28 (3H, d, J = 1.3, CH<sub>3</sub>C(3)), 2.40 (1H, ddd, <sup>2</sup>J = 14.2, <sup>3</sup>J = 5, <sup>4</sup>J = 1.8, H<sub>eq</sub>C(3')), 3.91 (1H, m, HC(4')), 5.98 (1H, d, J = 7.9, HC(2)), 6.40, 6.50 (2H, AB, J = 15.8, HC(5), HC(4)), 10.13 (1H, d, J = 8.0, CH(1); *m/z* (EI) 250 (M<sup>+</sup>, 9.4 %), 221 (6), 208 (3), 203(1) 191 (8), 175 (14), 168 (45), 149 (100), 133 (47), 123 (77), 107 (66), 95 (74), 77 (27), 67 (15).

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## **5. Appendix**

Selected  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR Spectra

Figure 2.  $^1\text{H}$  NMR spectrum of (24)

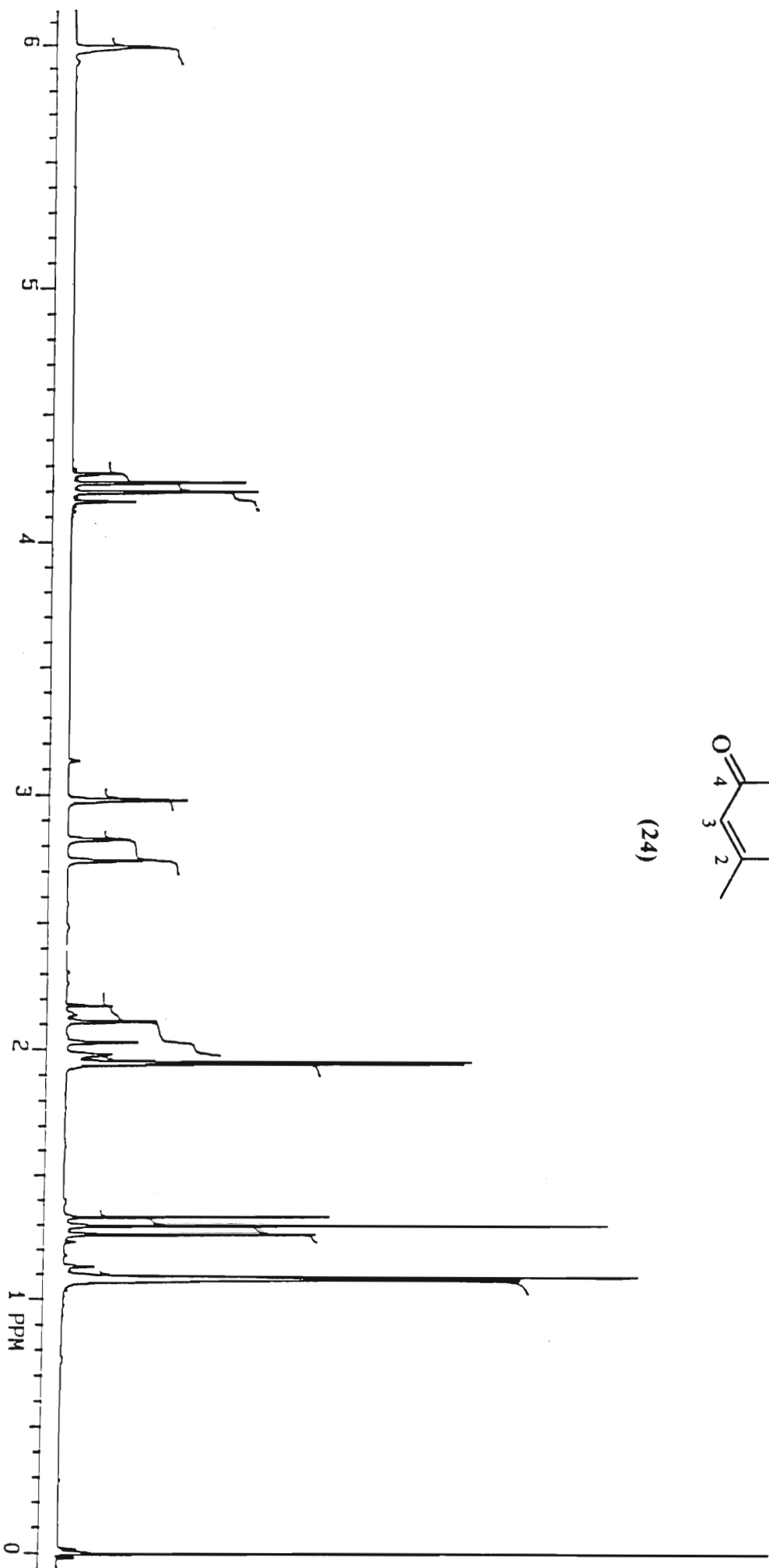
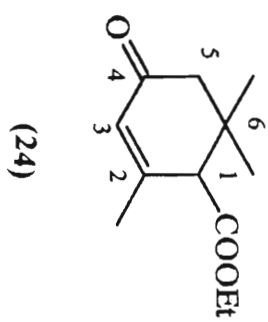


Figure 3.  $^{13}\text{C}$  NMR spectrum of (24)

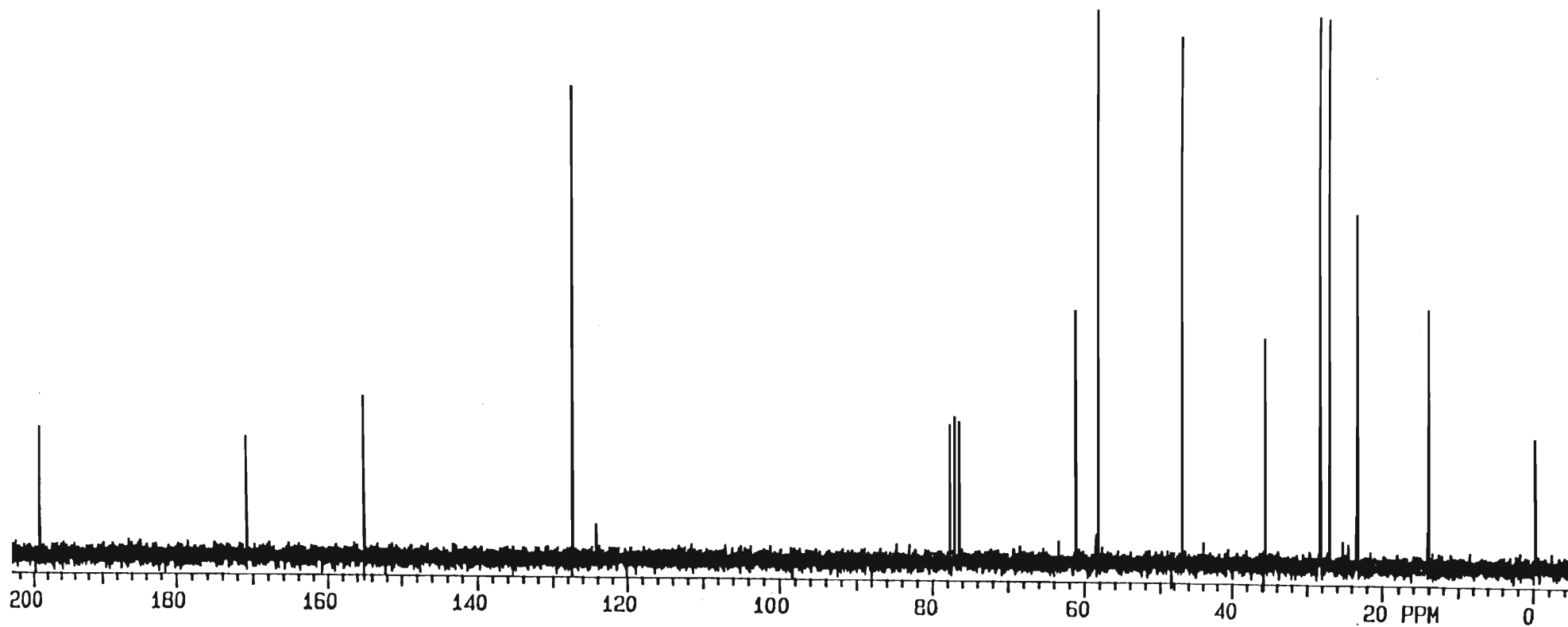
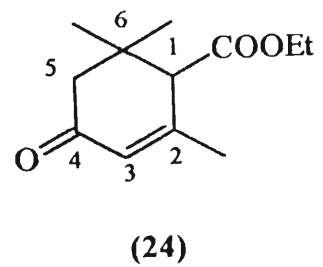


Figure 4.  $^1\text{H}$  NMR spectrum of (42)

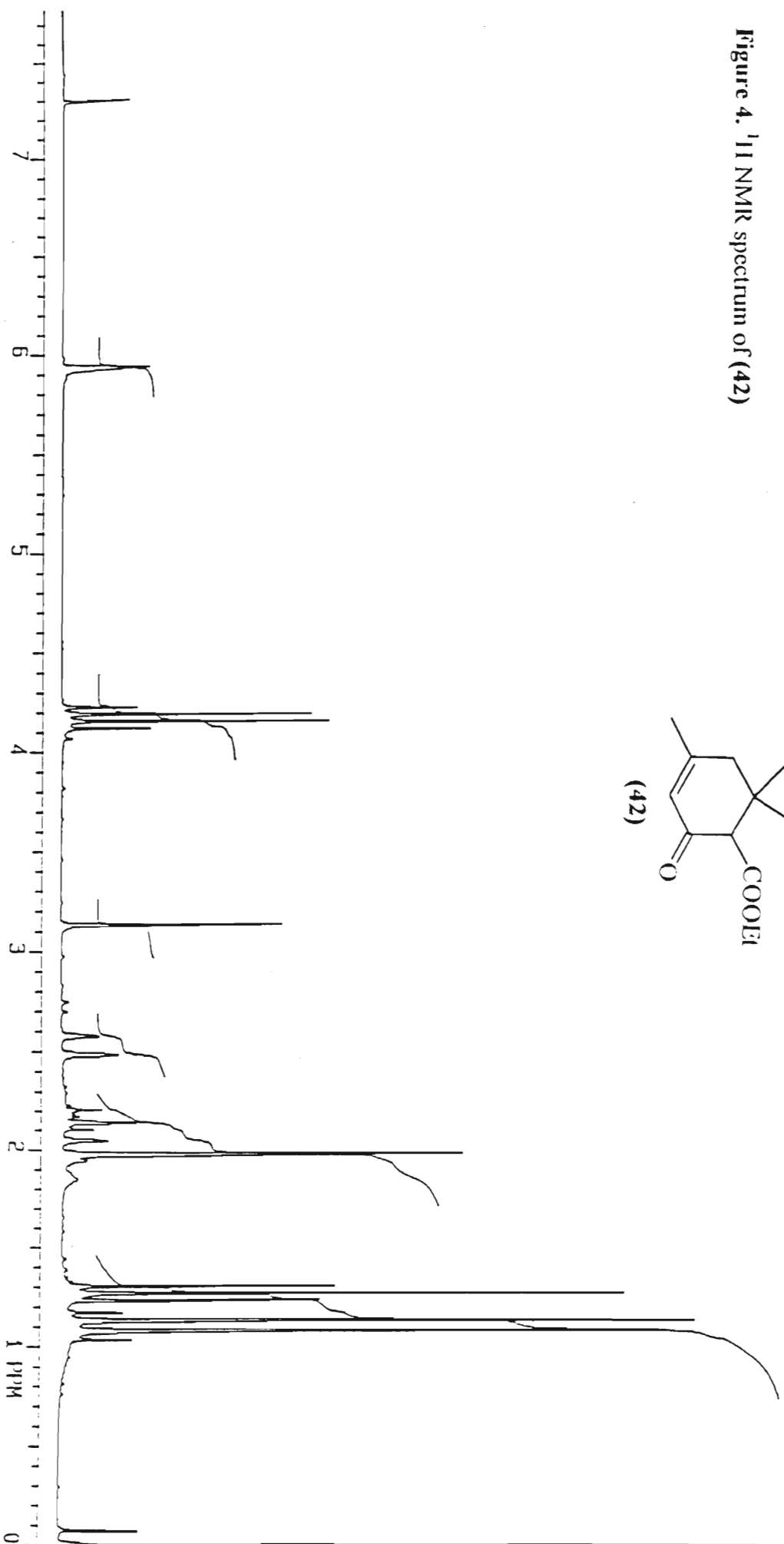
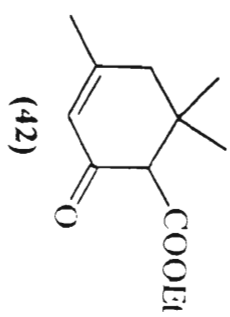


Figure 5.  $^{13}\text{C}$  NMR spectrum of (42)

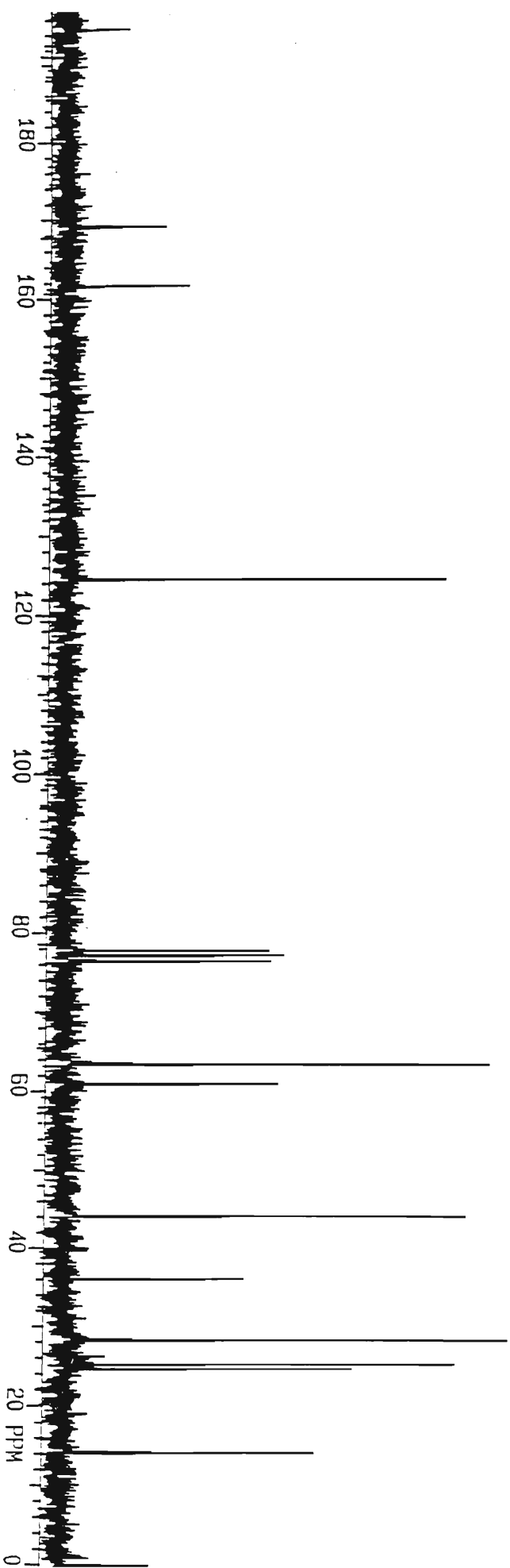
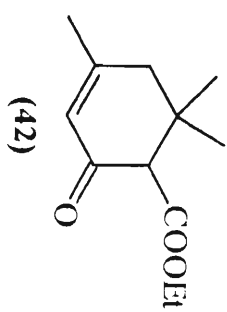


Figure 6. <sup>1</sup>H NMR spectrum of (25)

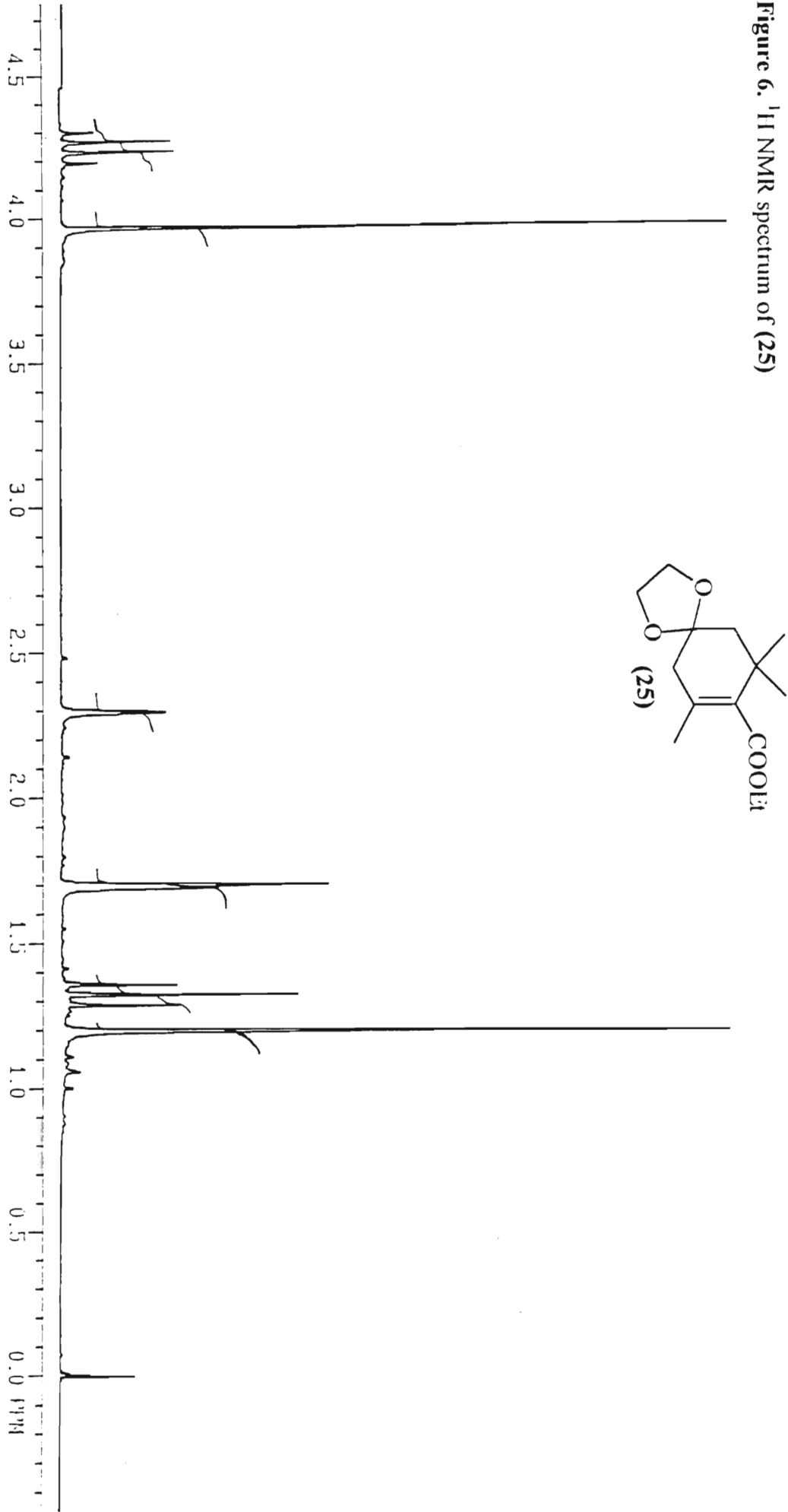


Figure 7.  $^{13}\text{C}$  NMR spectrum of (25)

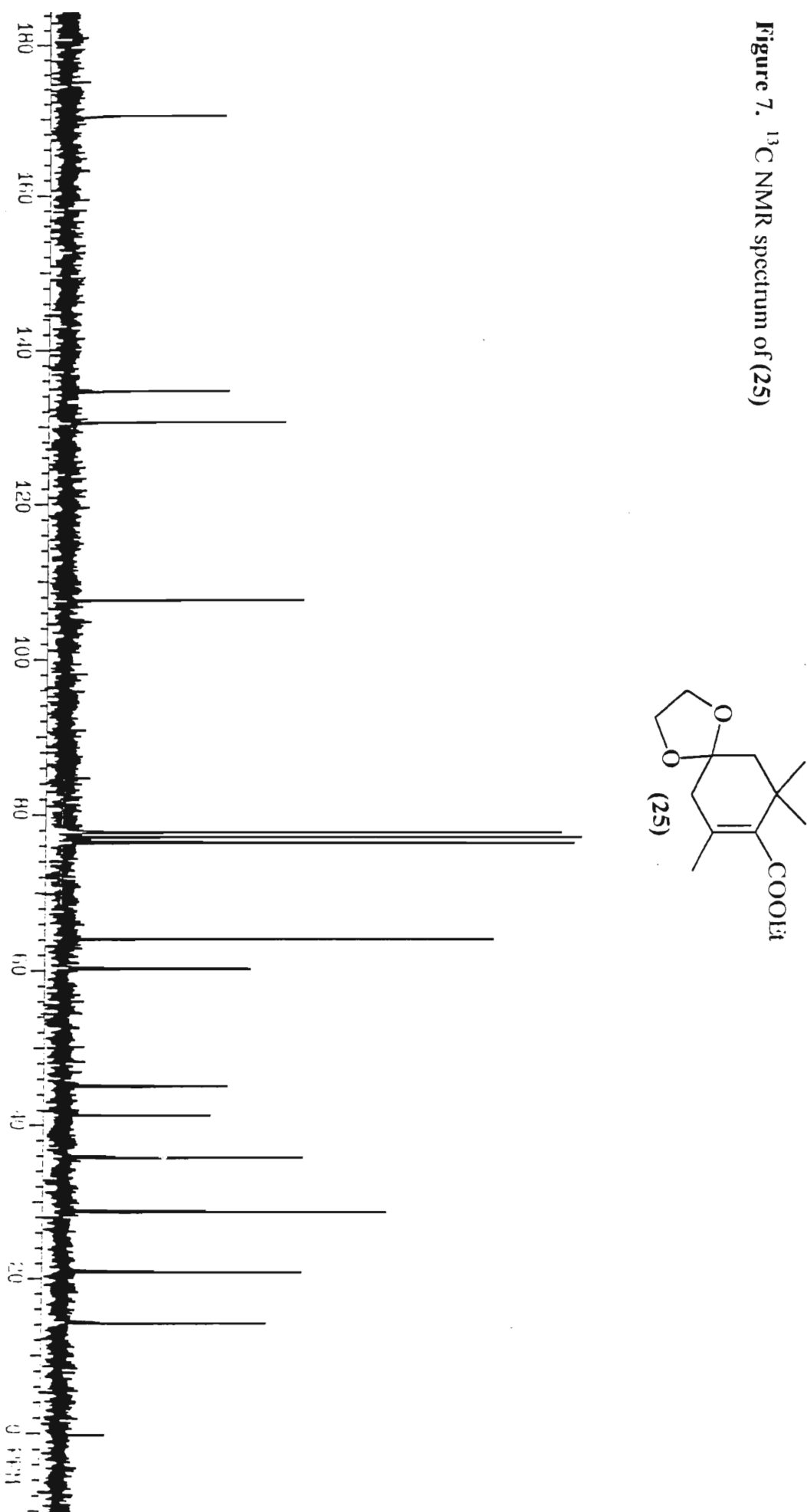


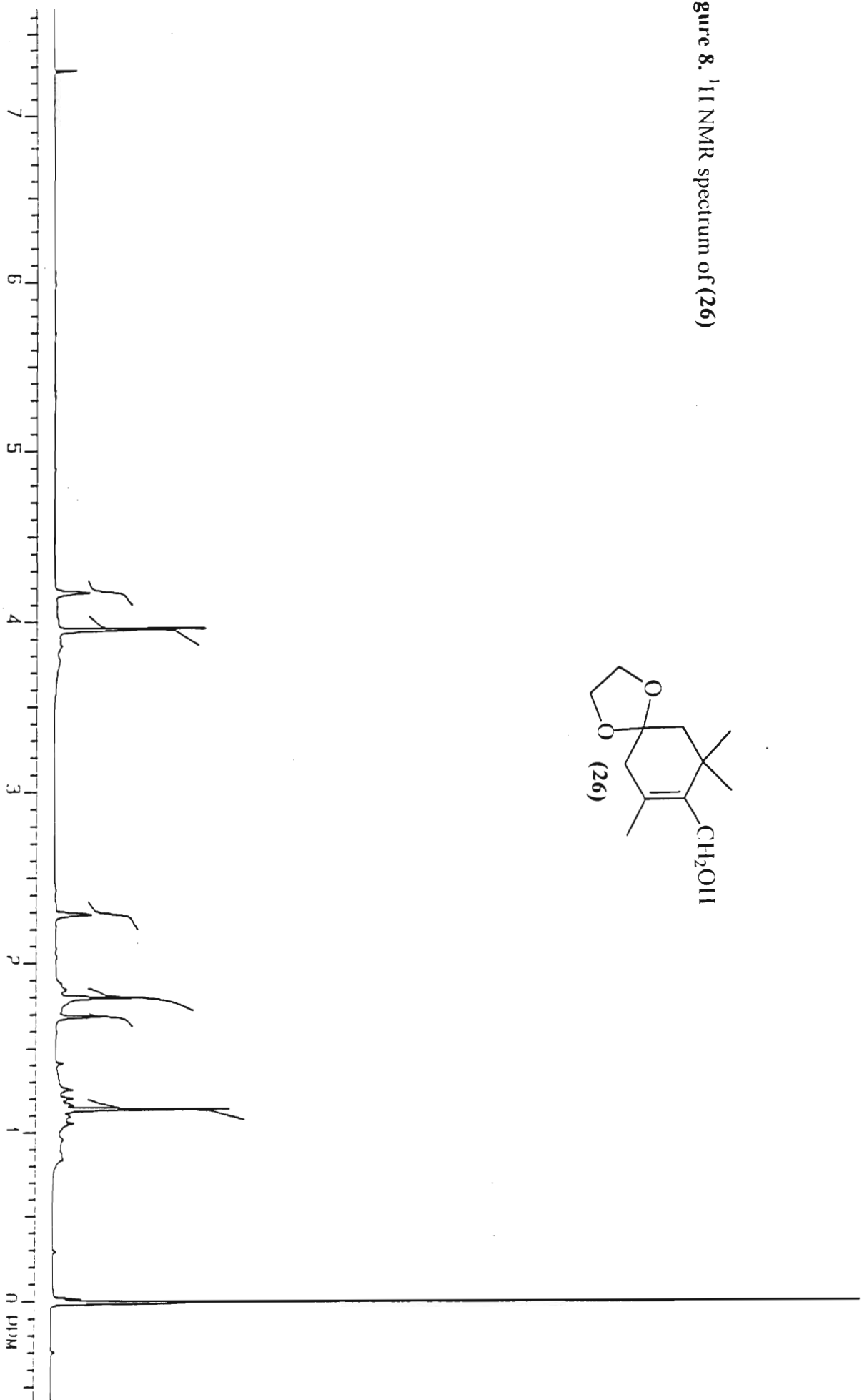
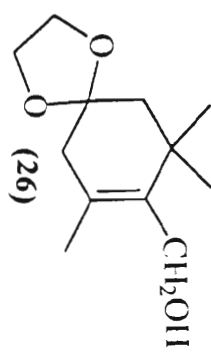
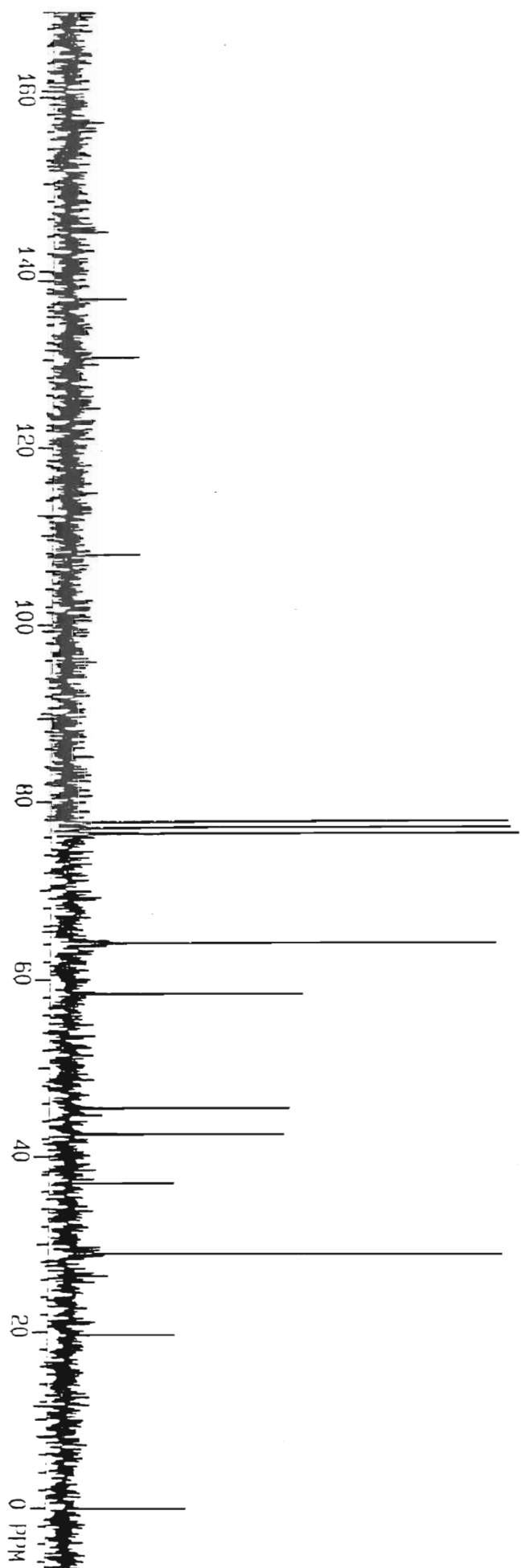
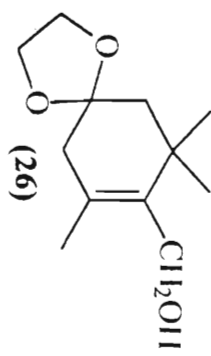
Figure 8.  $^1\text{H}$  NMR spectrum of (26)

Figure 9.  $^{13}\text{C}$  NMR spectrum of (26)



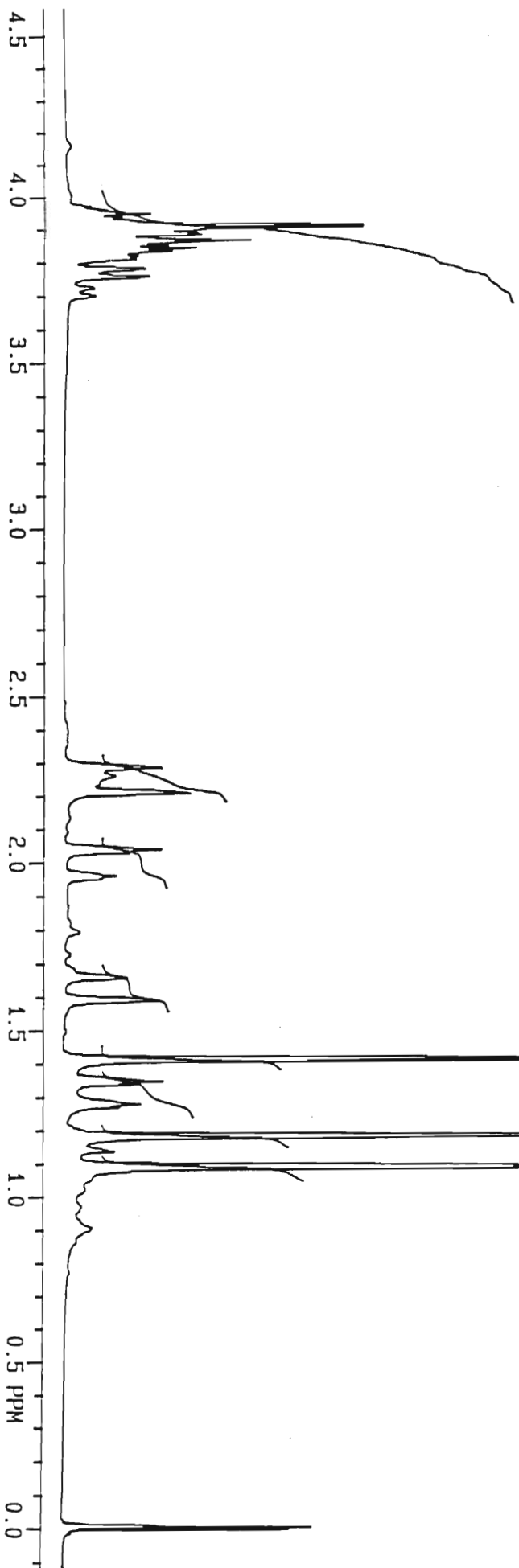
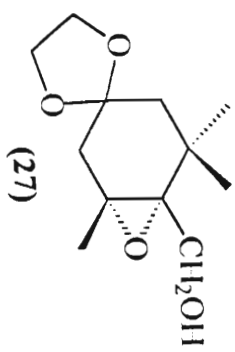
re 10.  $^1\text{H}$  NMR spectrum of (27)

Figure 11.  $^{13}\text{C}$  NMR spectrum of (27)

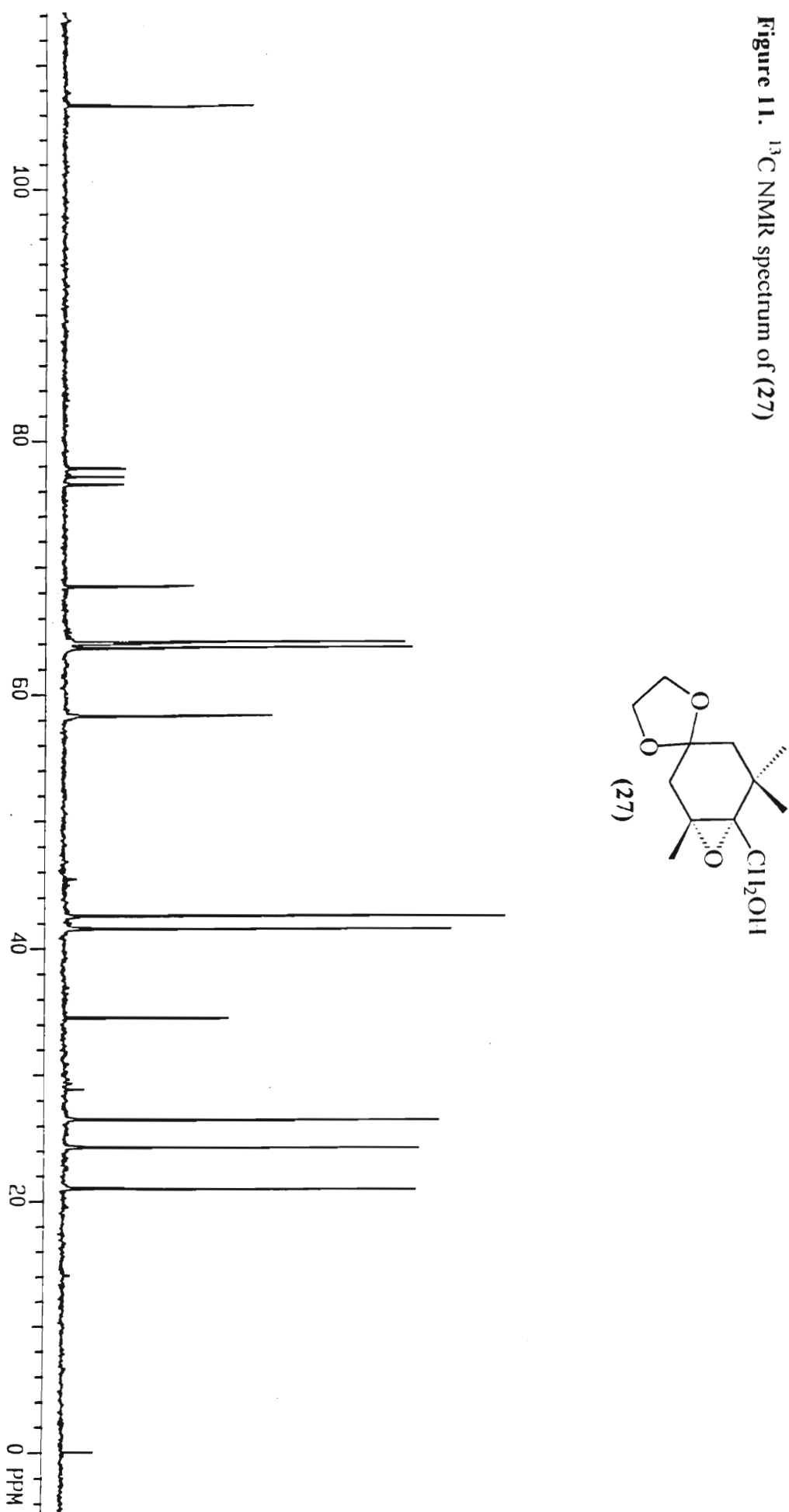


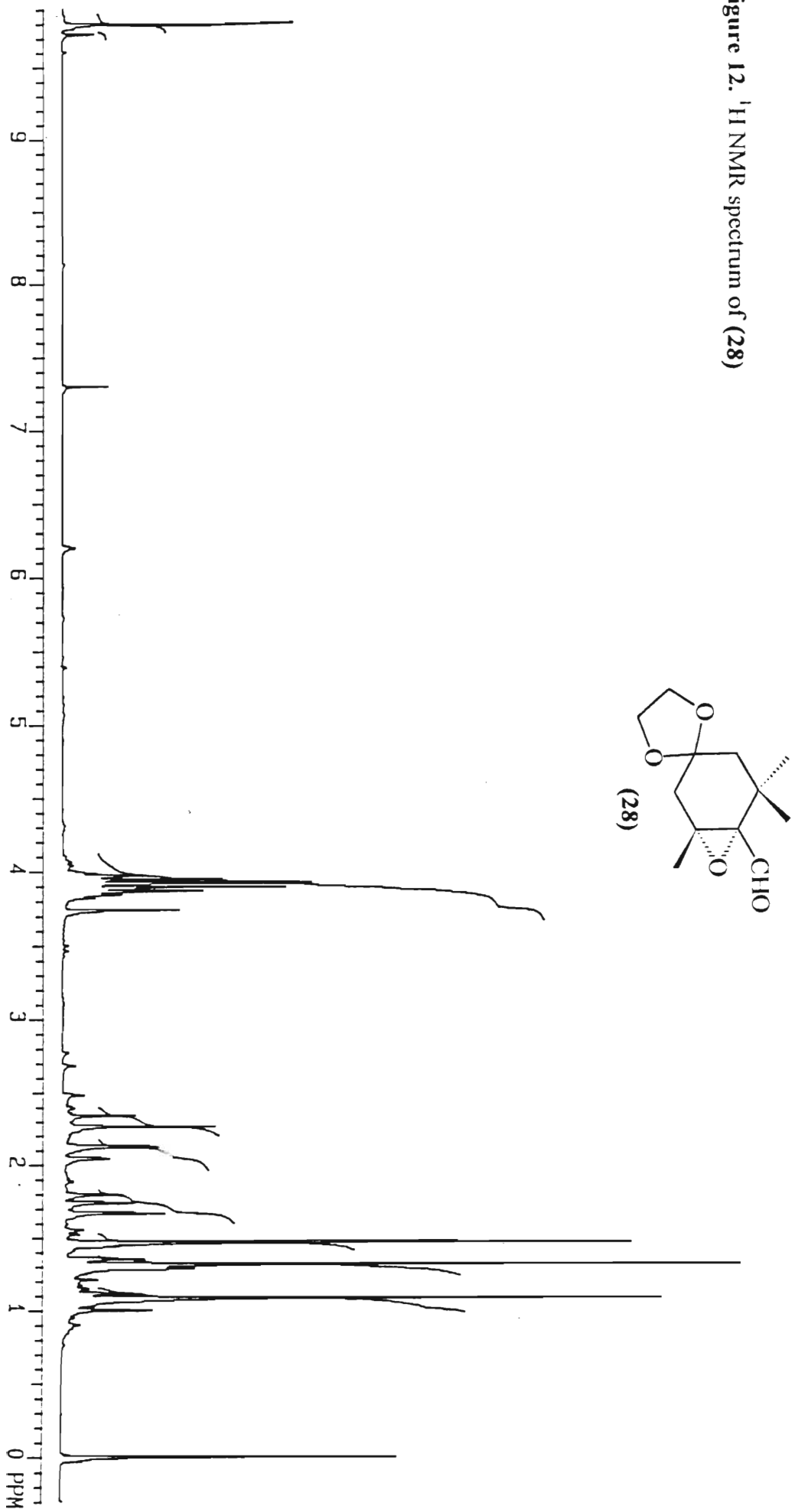
figure 12.  $^1\text{H}$  NMR spectrum of (28)

Figure I3.  $^{13}\text{C}$  NMR spectrum of (28)

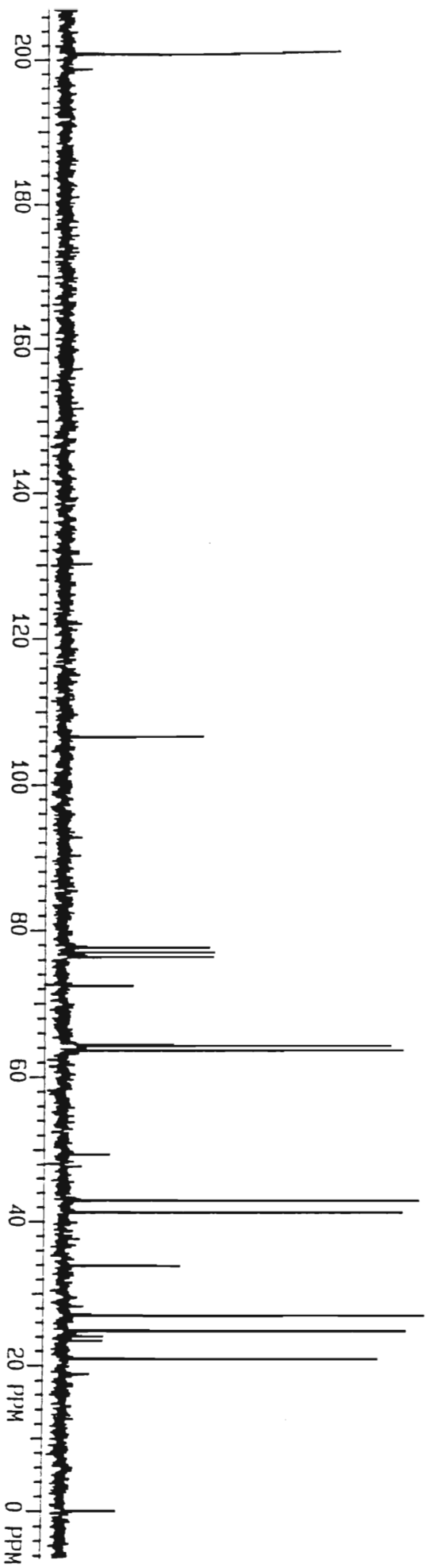
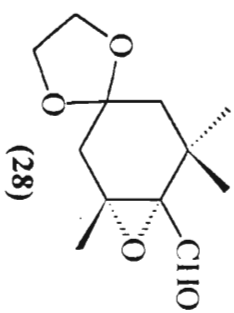


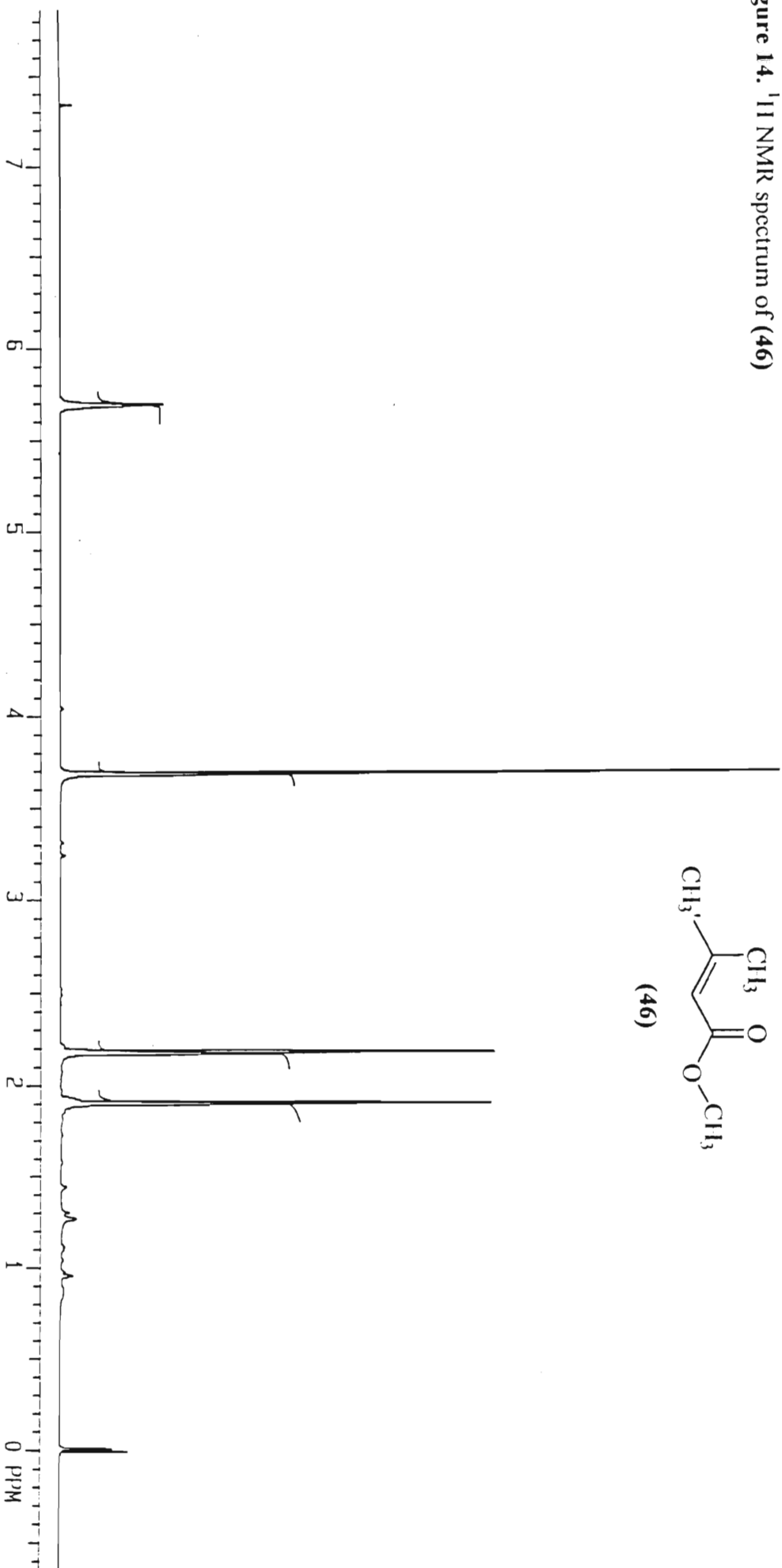
Figure 14.  $^1\text{H}$  NMR spectrum of (46)

Figure 15.  $^{13}\text{C}$  NMR spectrum of (46)

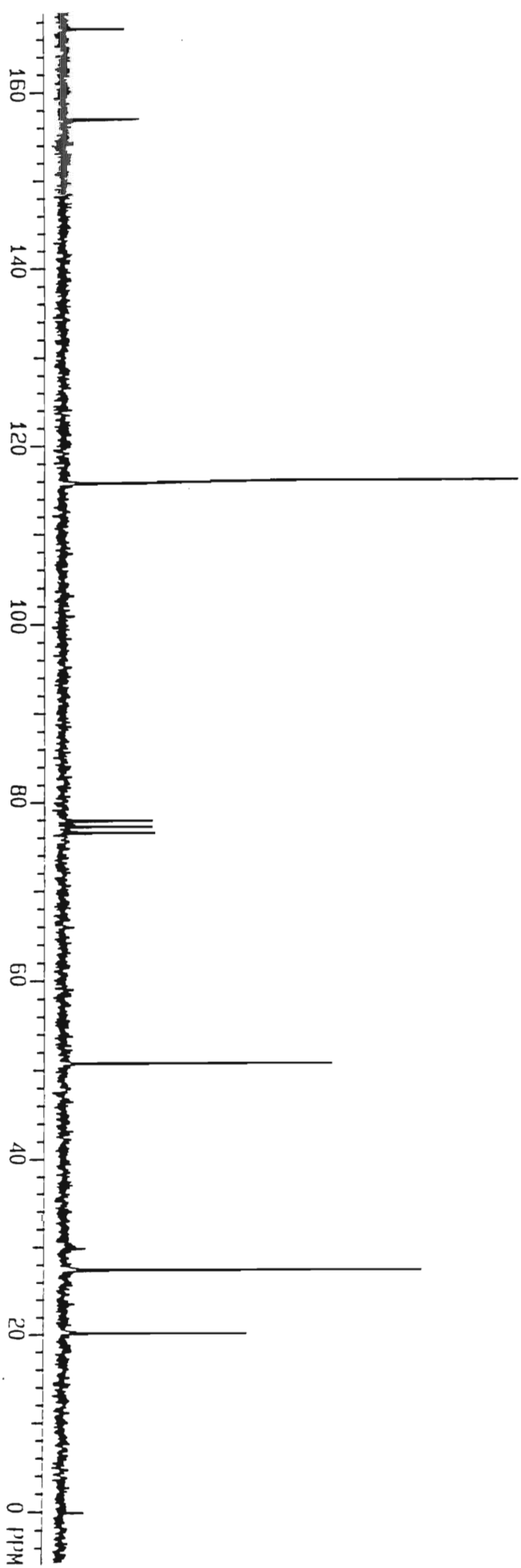
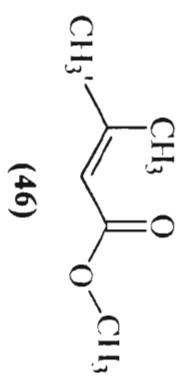
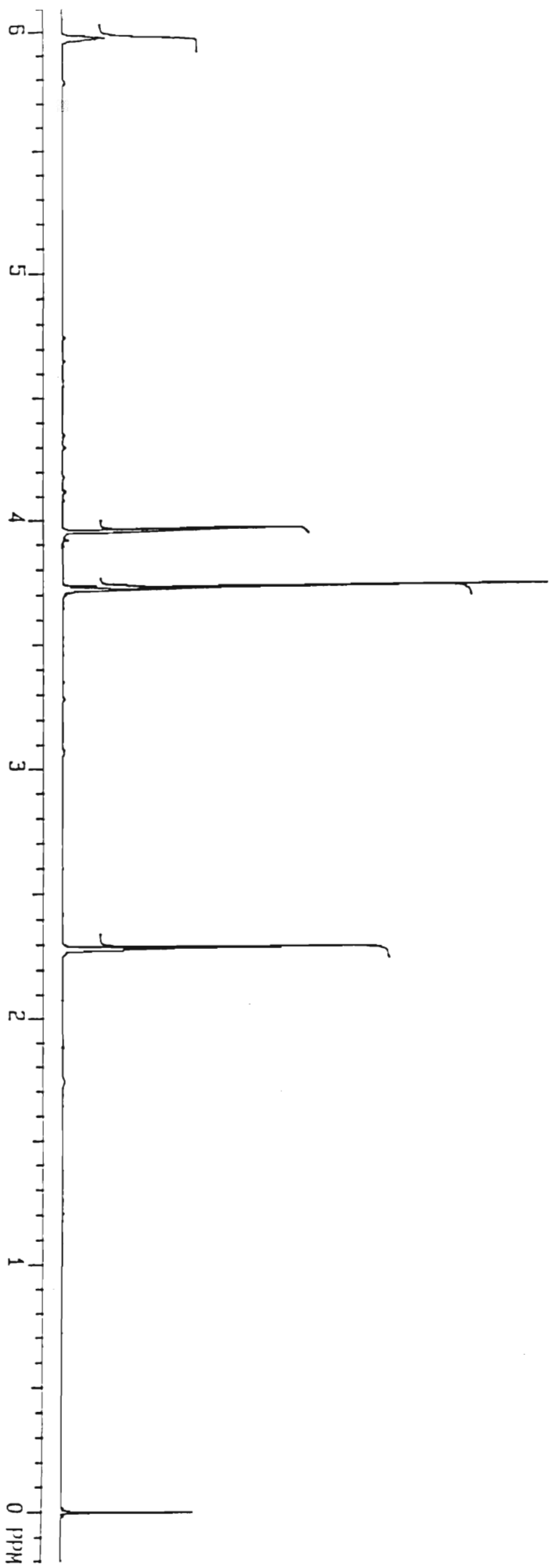
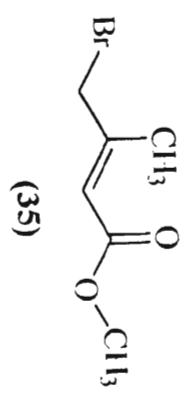


Figure 16.  $^1\text{H}$  NMR spectrum of (35)



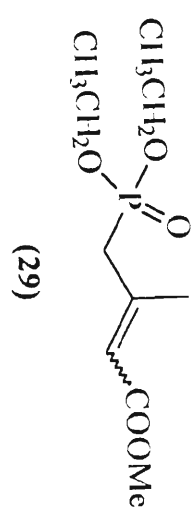


Figure 18.  $^1\text{H}$  NMR spectrum of (29)

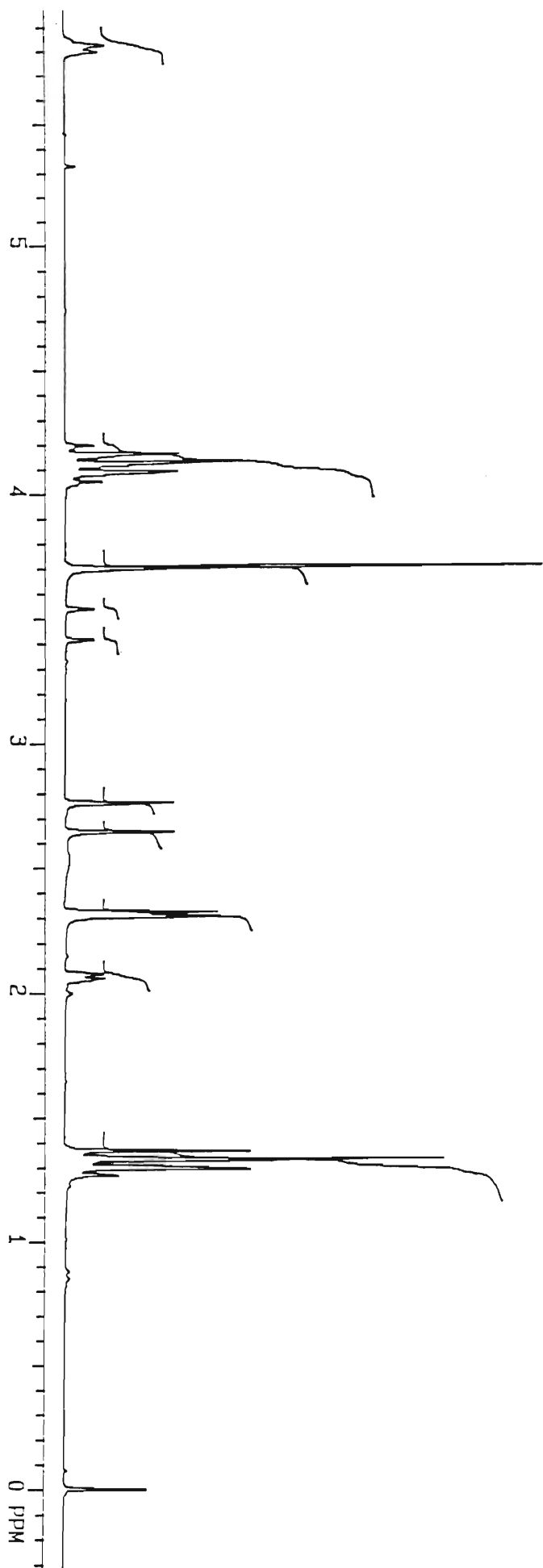


Figure 19.  $^{13}\text{C}$  NMR spectrum of (29)

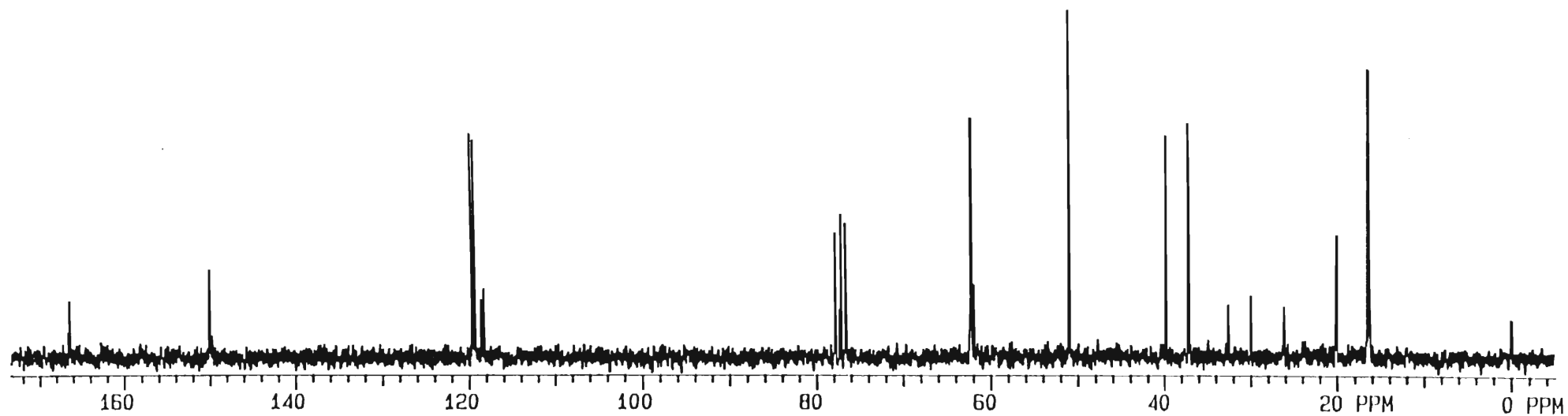
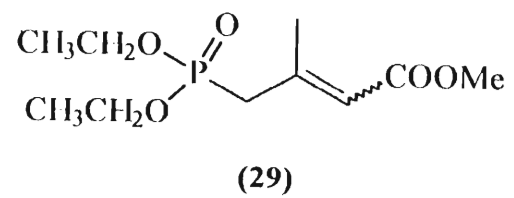


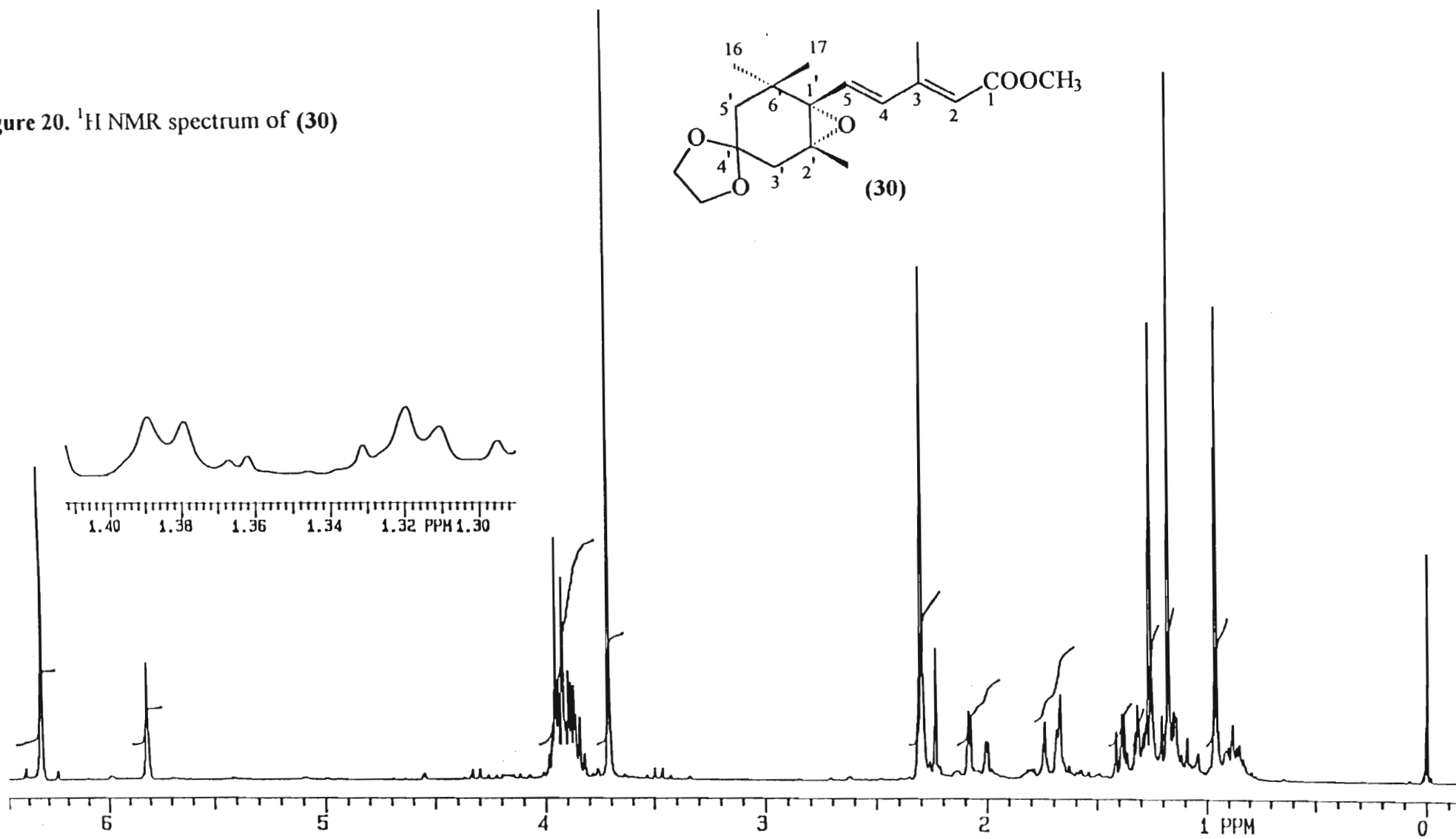
Figure 20.  $^1\text{H}$  NMR spectrum of (30)

Figure 21.  $^{13}\text{C}$  NMR spectrum of (30)

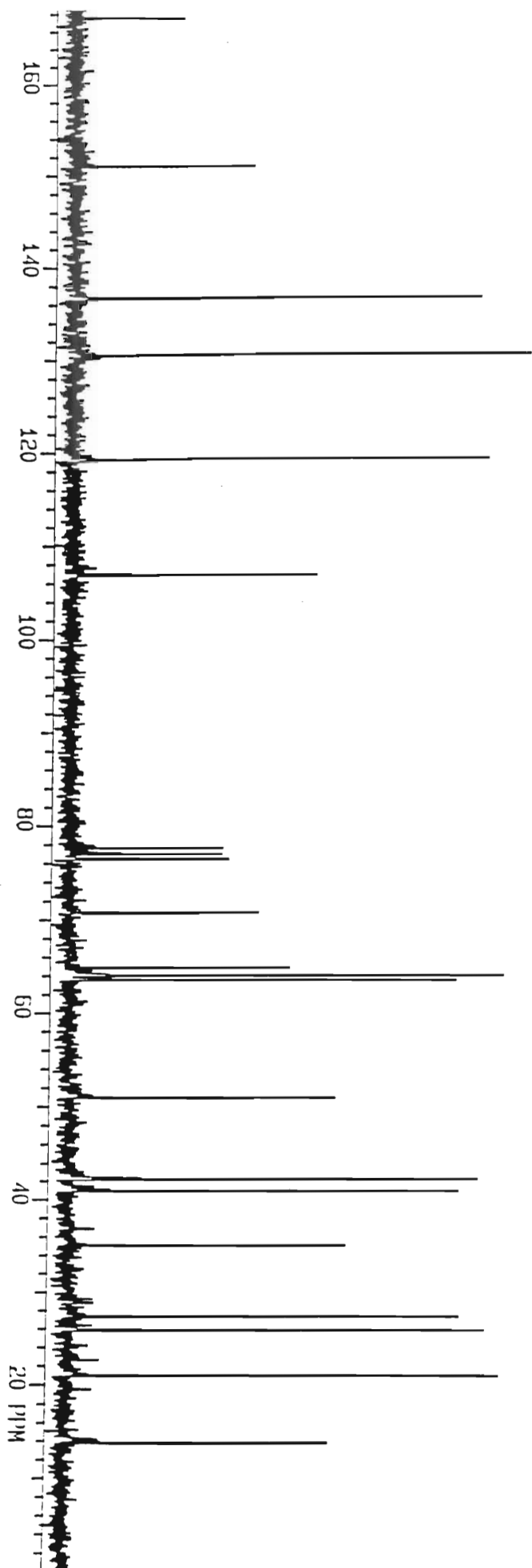
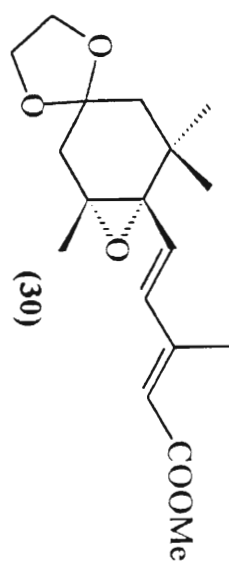


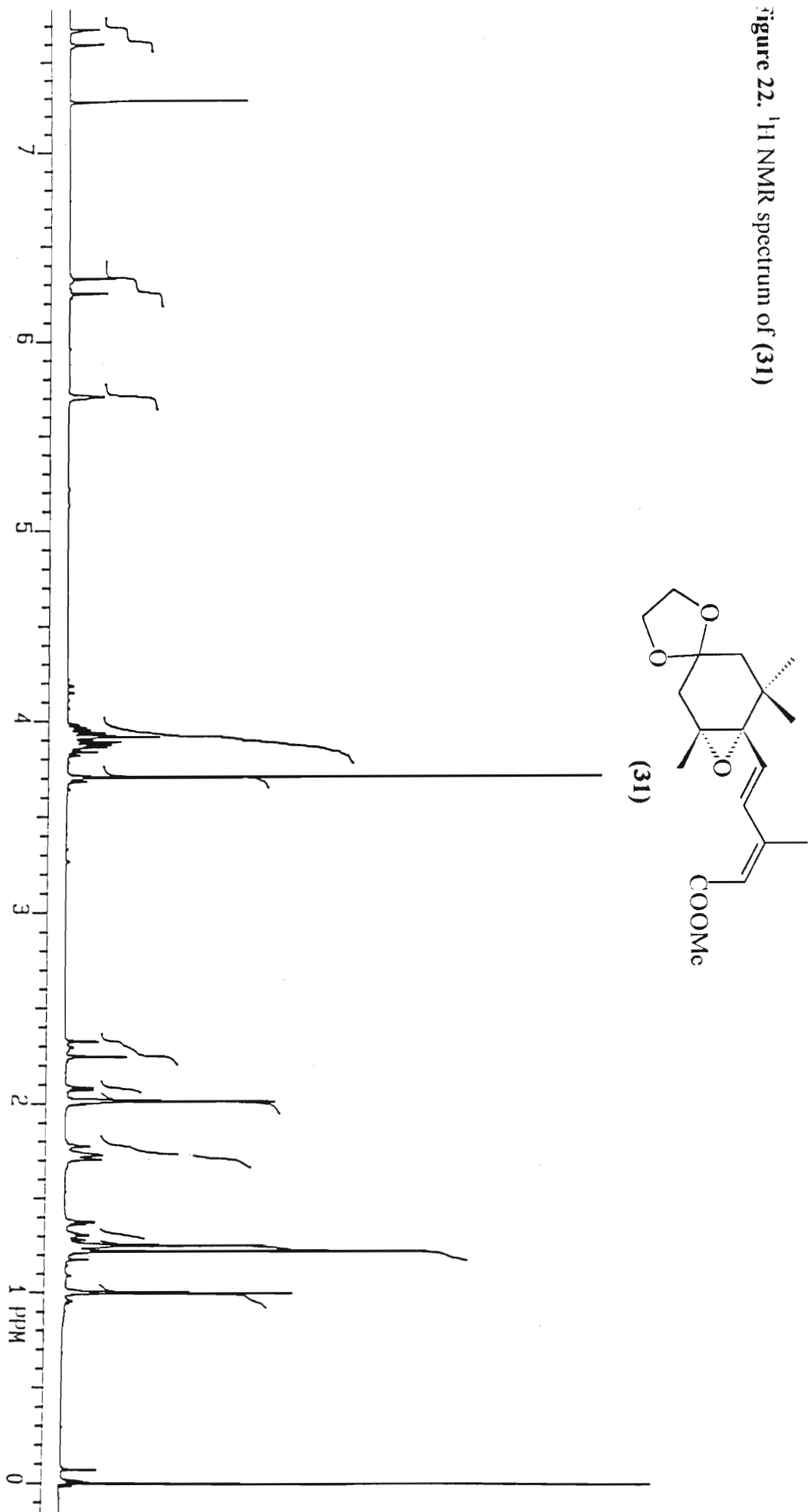
Figure 22.  $^1\text{H}$  NMR spectrum of (31)

Figure 23.  $^{13}\text{C}$  NMR spectrum of (31)

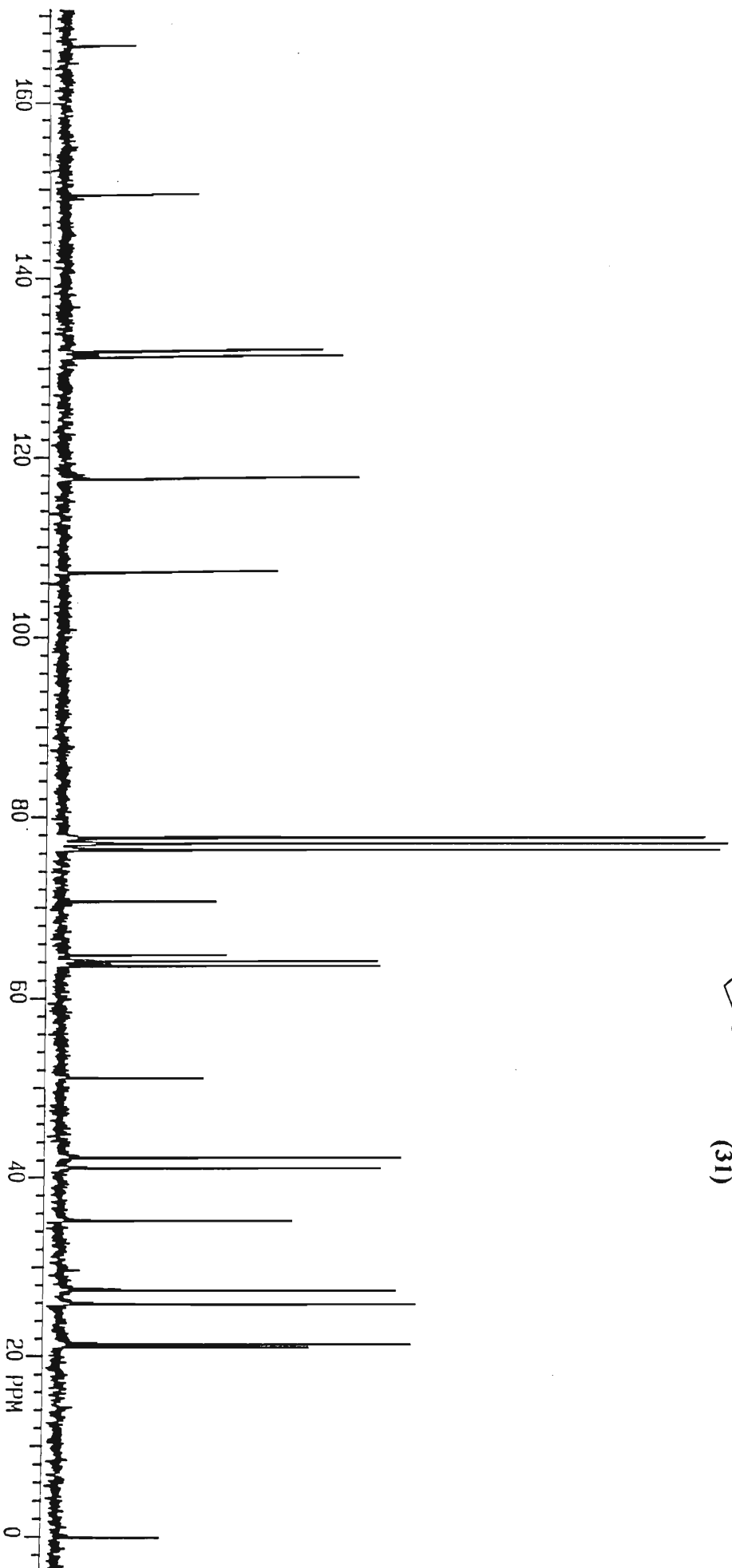
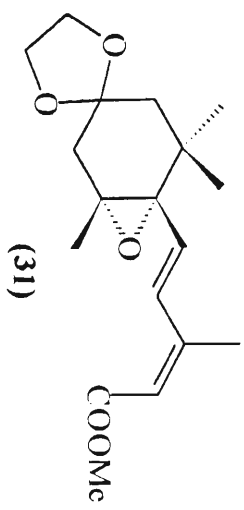
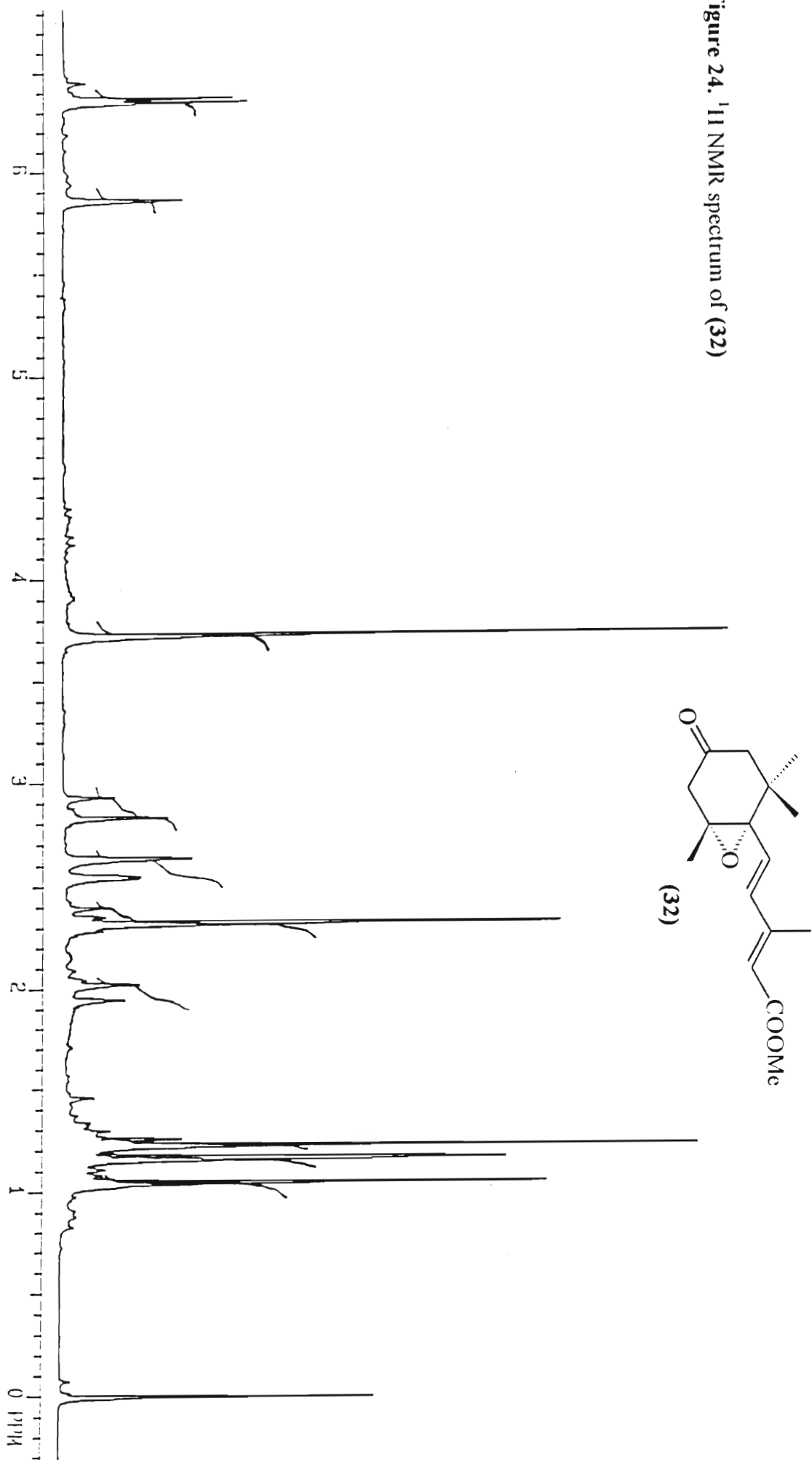


Figure 24.  $^1\text{H}$  NMR spectrum of (32)

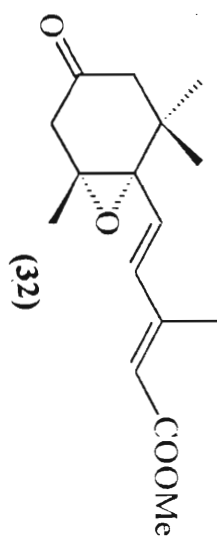


Figure 25.  $^{13}\text{C}$  NMR spectrum of (32)

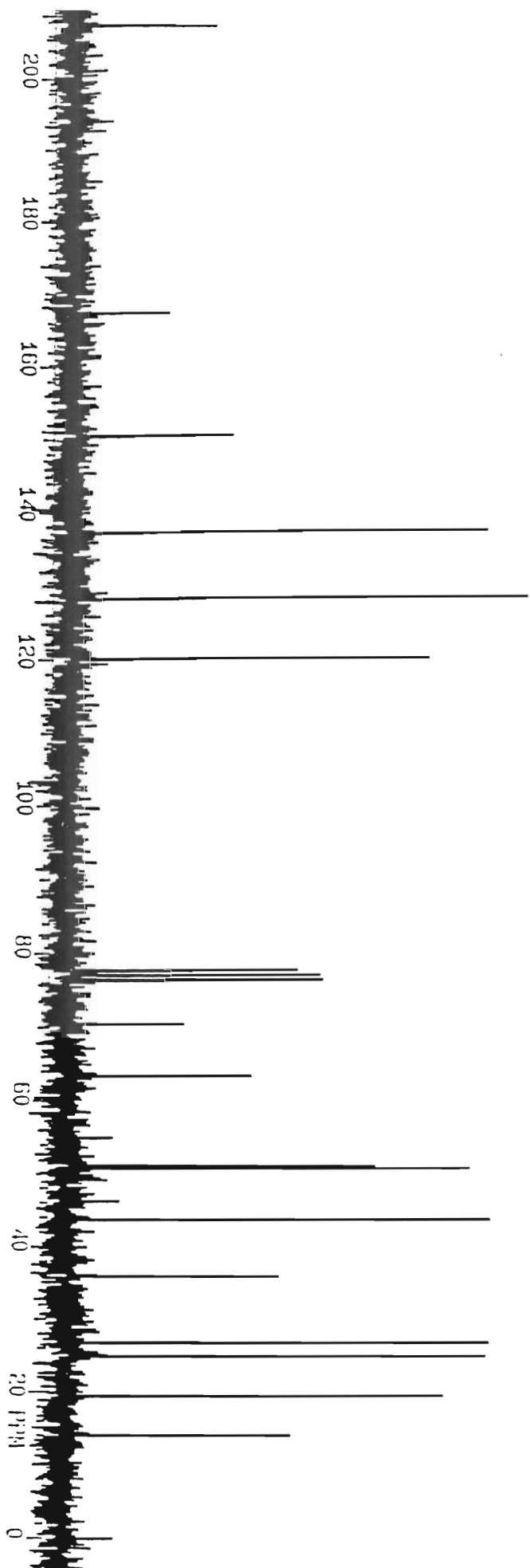


Figure 26.  $^1\text{H}$  NMR spectrum of (41)

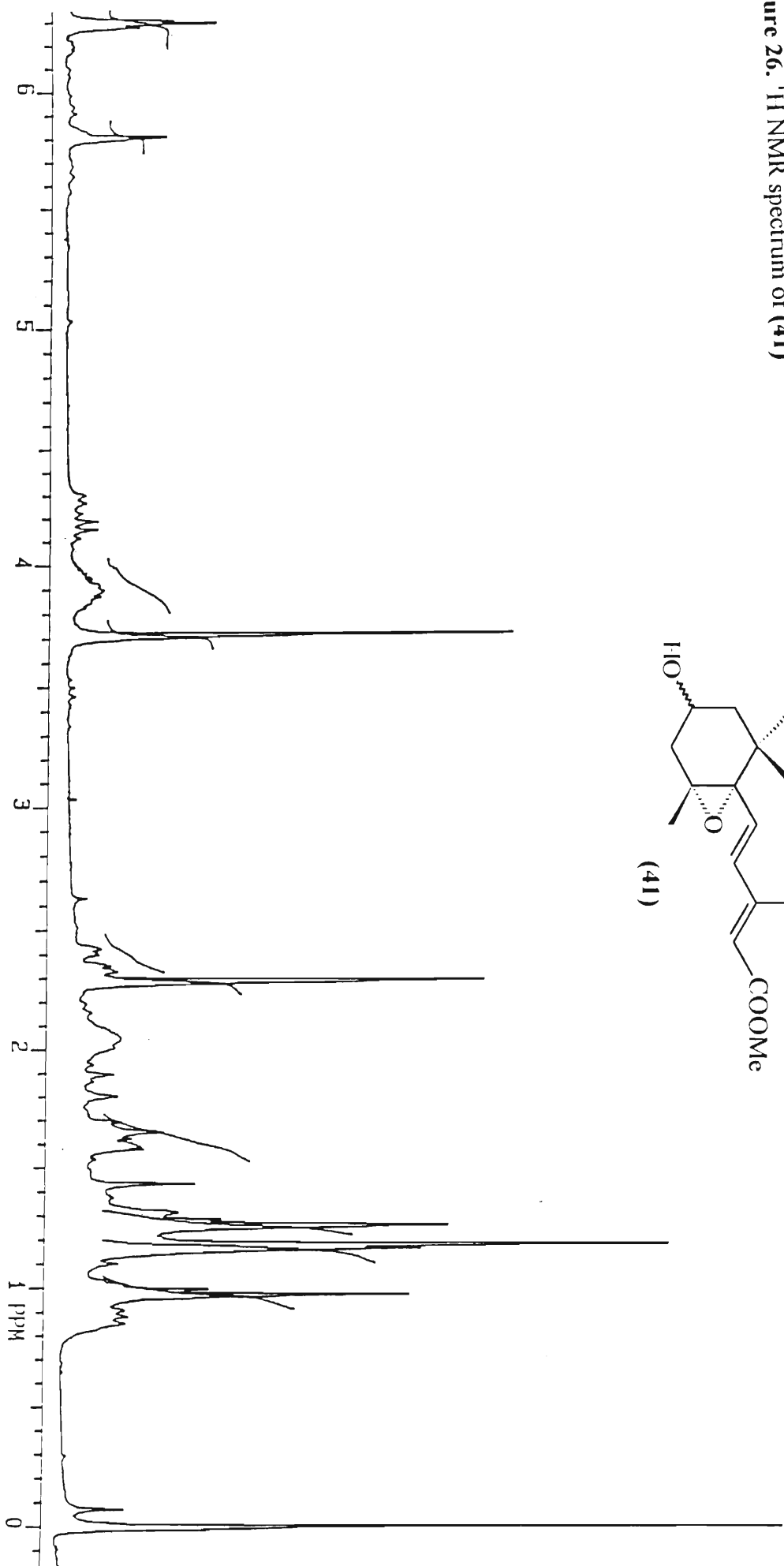
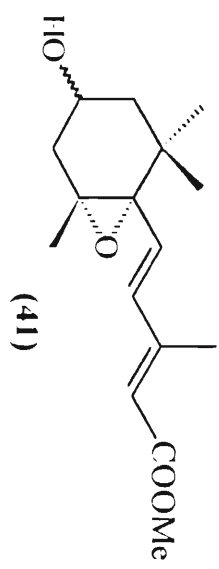


Figure 27.  $^{13}\text{C}$  NMR spectrum of (41)

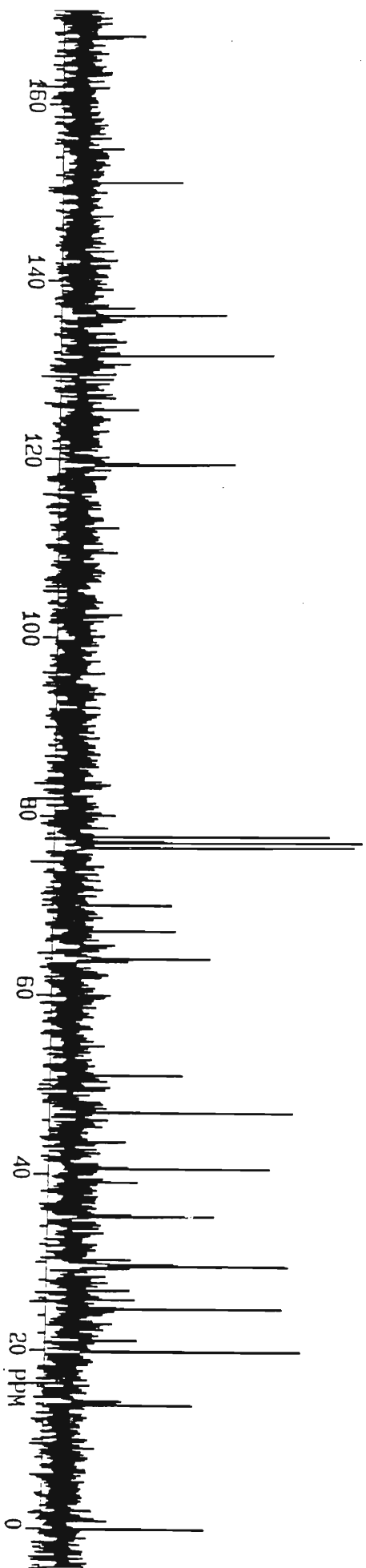
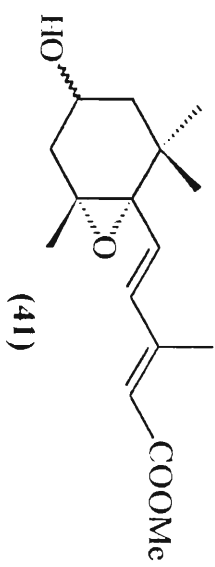


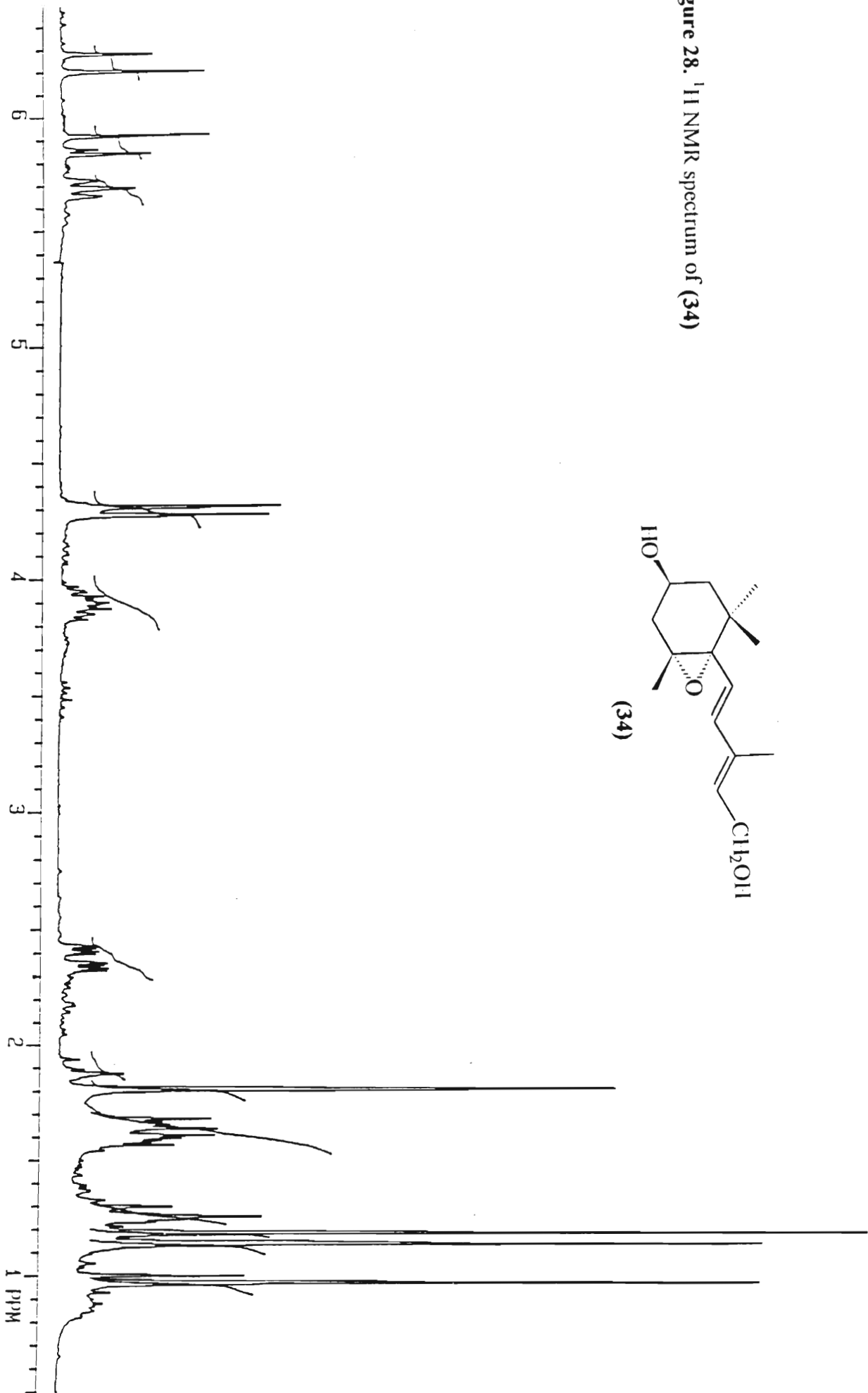
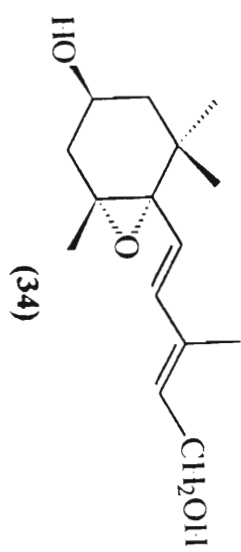
Figure 28.  $^1\text{H}$  NMR spectrum of (34)

Figure 29.  $^{13}\text{C}$  NMR spectrum of (34)

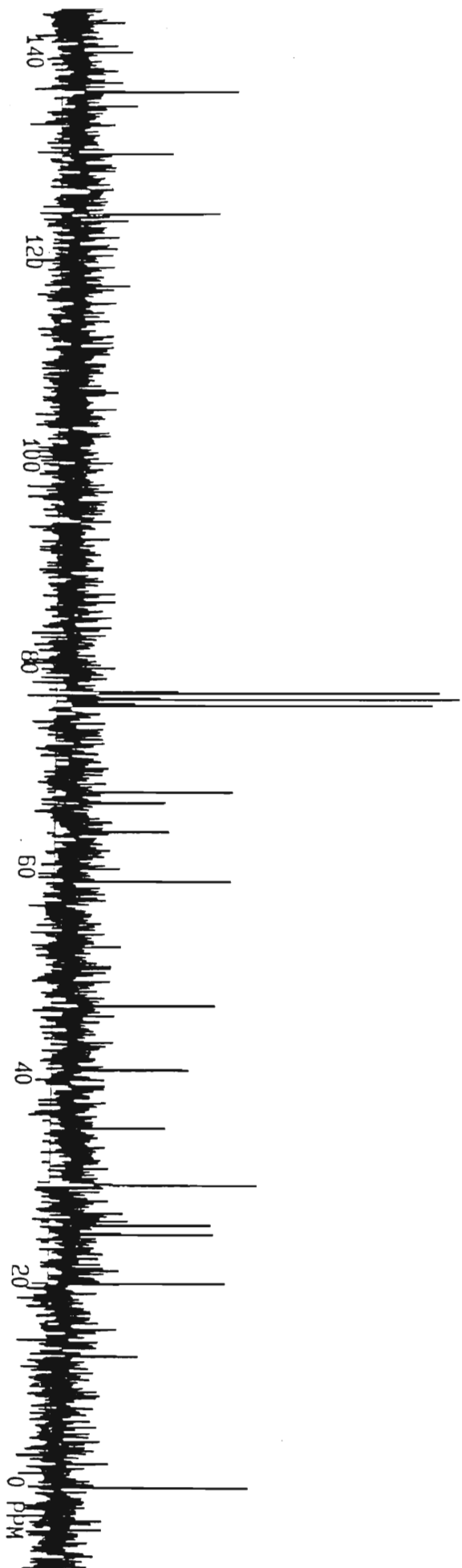
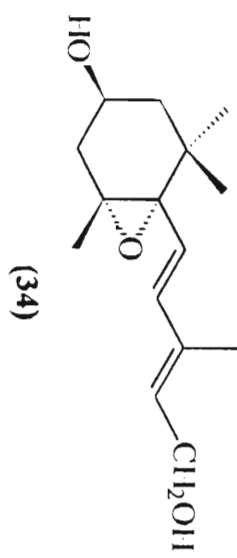


Figure 30.  $^1\text{H}$  NMR spectrum of (23)