The development of strategies for terpenoid structure determination

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Received (in Cambridge, UK) 18th July 2001
First published as an Advance Article on the web 18th September 2001

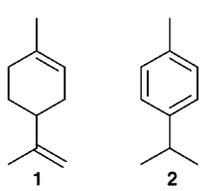
1 Introduction

It is the object of this review to illustrate the development of various strategies of terpenoid structure determination through the twentieth century. At the start of the century the methods were based on chemical degradation but by the end of the century they were dominated by the analysis of spectroscopic data. There are many examples which could be used to trace these developments. The constraints of space obviously limit the number that can be cited. It is important to note that the elucidation of the structure of a natural product may be the culmination of many years of effort in which the final solution to the problem may have come from the introduction of a new strategy. Nevertheless the structure would not have been established without the prior work. It is also worth reflecting on the extent to which natural product structure elucidation has influenced the impact of physical methods on organic chemistry in general.

Investigations into the structure of the monoterpenoids were well advanced by the end of the nineteenth century. The divisibility of their structures into isoprene units already formed a unifying feature.

The physical characteristics such as optical rotation and refractive index of various essential oils had been recorded by several workers. For example Gladstone had recorded[1] the characteristics of over fifty oils in 1864. The analysis of the hydrocarbons gave formulae of $C_{10}H_{16}$ for oil of turpentine (mainly α-pinene) and $C_{15}H_{24}$ for cedar wood oil (mainly cedrene). However, informative chemical degradation and structure elucidation required the characterization of the individual components of these oils. An important advance in this context was the introduction of nitrosyl chloride by Tilden in 1875[2] as a reagent for the formation of crystalline derivatives of terpenes, for example, α-pinene from oil of turpentine. This served to distinguish one terpene from another.

Thermal decomposition of the vapour of oil of turpentine was shown by Tilden in 1884[3] to give isoprene (C_5H_8). Isoprene had been obtained previously by the distillation of rubber and it was known to dimerize to dipentene,[4] the dihydrochloride of which was identical to that of another monoterpene, limonene **1**. *p*-Cymene **2** was also obtained from these experiments. The presence of an isopropyl group in *p*-cymene and the structure of isoprene itself were not unambiguously established until 1891[5] and 1897.[6] Nevertheless as a guiding principle for structure elucidation, Wallach developed the theory in 1887,[7] that the monoterpenes were built up of two, the sesquiterpenes of three and the diterpenes of four isoprene units. He also suggested that whereas the simple terpenes had a skeleton based on *p*-cymene, the sesquiterpenes might be related to naphthalenes and the diterpenes to phenanthrenes.



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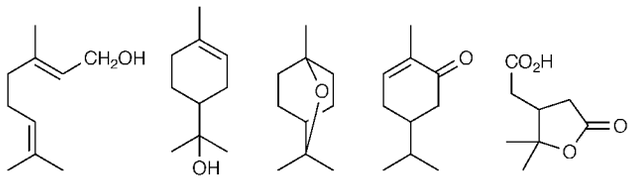
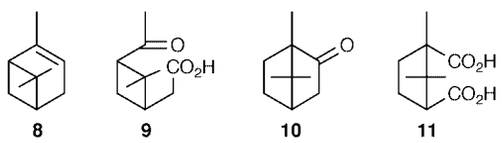
2 Oxidative degradation of monoterpenoids

The many studies of Wallach, Wagner, Tiemann and Semmler during the 1890's led to the correct structures of geraniol **3**, limonene **1**, α-terpineol **4**, 1,8-cineole **5** and carvone **6**. The structure of geraniol and linalool and hence of citral was suggested[8] in 1895 on the basis of the degradation *via* methylheptenone. The structures of limonene **1**[9] and α-terpineol **4**[10] were proposed on the basis of oxidative evidence utilizing potassium permanganate and chromic acid. The identification[11] of terpenylic acid **7** played an important role in these investigations. Limonene **1** was interrelated[12] with carvone **6** through the identification of nitrosolimonene with carvone oxime.

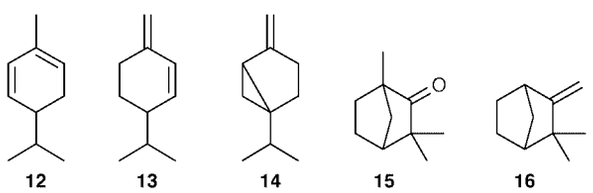
Although the subject of some dispute, the correct formula for α-pinene **8** was proposed in 1894.[13] Support was provided by the oxidation of α-pinene with potassium permanganate to pinonic acid **9** and its further degradation.[14] The structure of



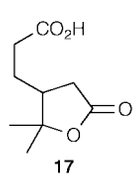
camphor **10** was established on the basis, *inter alia*, of the structure of the nitric acid oxidation product **11**.[15] The characteristic feature of all these studies was the reliance on the results of vigorous oxidative degradation to give identifiable crystalline products, typically acids and lactones. Hence the structures of many of the major monoterpenes had been established by the turn of the century.



The first decade of the century saw the establishment of the structures of further monoterpenes such as the α- and β-phellandrenes **12** and **13**,[16] sabinene **14**,[17] fenchone **15** and camphene **16**[18] whilst the stereochemical relationships between geraniol, nerol and the citrals a and b were elucidated.[19]

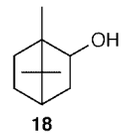


An important facet of this work involved the unambiguous synthesis of the oxidative degradation products of the terpenes such as camphoric acid **11**,[20] α-campholactone,[21] terpenylic **7** and homoterpenylic acids **17**.[22] Terpineol was synthesized[23] in 1904 by a route which was subsequently extended to a series of menthenols, menthadienes and menthones. Whilst to modern eyes these syntheses may appear simple, they are nevertheless notable for their reliance on relatively few types of reactions including base-catalysed Claisen condensations, alkylation and Grignard reactions. They provided the confirmatory evidence for the structures of the monoterpenes.



3 The Wagner–Meerwein rearrangement

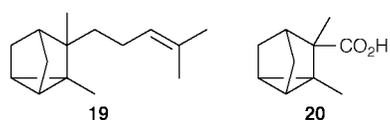
The structure of camphene **16** and its relationship to borneol **18** had been a puzzle for much of the first decade of the twentieth century. Wagner had suggested[24] in 1899 that the conversion of borneol to the camphene skeleton could be regarded as a rearrangement. In 1914 Meerwein brought forward further evidence to support the view.[25] Mechanistic studies on the Wagner–Meerwein rearrangement were reported[26] in the early 1920's to show that tricyclene was not an intermediate and established the ionic nature of the rearrangement of bornyl chloride to camphene. Hence the idea that aspects of terpenoid chemistry could be rationalized in terms of carbocation formation was already current at this time.



4 Early studies on the sesqui- and diterpenoids

During the first decade of the century, work on the sesqui- and diterpenoid natural products was less successful. With hindsight the nature of the ring systems, and the existence of closely related isomers, made several of the examples that were investigated (*e.g.* caryophyllene from oil of cloves) unfortunate choices.

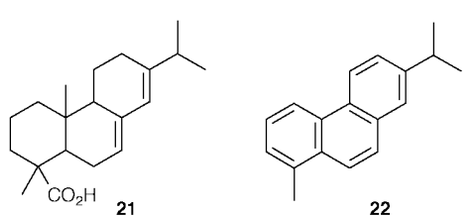
Investigations by Semmler into the structures of the santalenes from sandalwood oil commenced in 1907.[27] The stepwise degradation of α-santalene **19** to teresantalic acid **20** enabled Semmler to propose a structure for the sesquiterpene in 1910.[28] A structure for farnesol was proposed in 1913.[29]



5 The development of dehydrogenation as a structural method

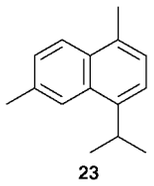
Two major developments in the early 1920's, both associated with the work of Ruzicka, had a major impact on the elucidation of the structures of the sesqui- and diterpenes. The first was the development of dehydrogenation[30] as a structural tool and the second was the application of the isoprene rule[31] in evaluating possible terpenoid structures.

The dehydrogenation of abietic acid **21** with sulfur to give retene (1-methyl-7-isopropylphenanthrene) **22** had been reported by Vesterberg in 1903.[32] However it was the work of Ruzicka which developed this into a major method of structure elucidation. The dehydrogenation of sesqui- and diterpenoids with sulfur and later selenium to give aromatic compounds provided a useful structural simplification in that it eliminated stereochemical problems particularly at ring junctions, and yet it afforded in many cases a crystalline alkylated aromatic hydrocarbon retaining the connectivity of the majority of the carbon atoms of the parent terpenoid. These compounds were readily characterized by their crystalline derivatives and the aromatic hydrocarbons could be synthesized by methods which unambiguously located their alkyl substituents. Initially two naphthalenes, cadalene **23** (1,6-dimethyl-4-isopropylnaphthalene) and eudalene **24** (1-methyl-7-isopropylnaphthalene) were obtained[30,33] from cadinene **25** and selinene **26**, respectively. Sesquiterpenes were at first grouped on the basis of these naphthalenes.



6 The isoprene rule

Examination of the structures of these naphthalenes led[34] to the idea that the sesquiterpenes might be derived by the initial union of the isoprene units to form farnesol whilst geraniol could give *p*-methylisopropylbenzene (*p*-cymene) characteristic of the monoterpenes. The head-to-tail union of isoprene units and the folding of the farnesol chain to generate the cadinane



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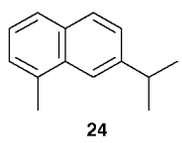

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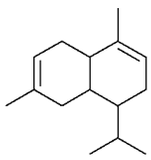

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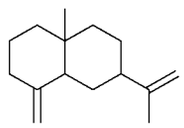

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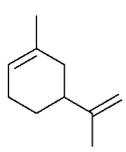
and eudesmane skeleta was widely used in the evaluation of sesquiterpenoid structures.[35]

However there were a number of apparent exceptions to this rule. The natural occurrence of the monoterpene, sylvestrene **27**, raised some interest. Although its structure could be dissected into isoprene units, it cannot be derived by the simple cyclization of geraniol. A careful re-examination of *Pinus sylvestris* revealed[36] the presence of Δ^3-carene **28** rather than sylvestrene. The latter had previously been isolated from the oil by treatment with hydrogen chloride. The demonstration of the absence of sylvestrene from this oil removed a major exception to the view that the isoprene–geraniol union constituted the first step in the formation of monoterpene structures.



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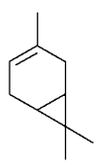

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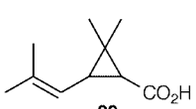
Structural work on the insecticidal constituents (*e.g.* **29**) of *Chrysanthemum cinerariifolium*, although carried out during the period 1910–1916, was not published[37] until 1924 and furnished together with lavandulol **30** a further group of substances which whilst containing isoprene units did not appear to follow a regular isoprene rule.



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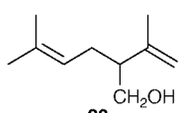

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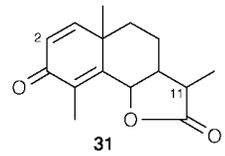
The elucidation of the structure of santonin **31** exemplified the application of the rule. Santonin is an anthelmintic which was obtained from the flowers of *Artemisia maritima*, and it had been the subject of investigation since the 19th century. These investigations had revealed its interesting photochemistry as well as the formation of the phenolic desmotroposantonins **32**. Indeed a structure representing the ketonic tautomer of a phenol had been proposed[38] for santonin in 1892. Confusion had occurred because it was not realized that rearrangement of the methyl group from the angular position had taken place during the degradation. An alternative structure taking this into account and based on the principle of the head-to-tail linkage of isoprene units was made in 1929.[39] The synthesis of santonous acid **33** and a proof of the angular position of a methyl group, led to the proposal of the overall structure **31** in 1930.[40]

Structural work on α-cyperone **34**,[41] which was obtained from the oil of *Cyperus rotundus*, was typical of the structural methodology current at this time. Dehydrogenation with selenium gave 1-methyl-7-isopropylnaphthalene **24**. The location of



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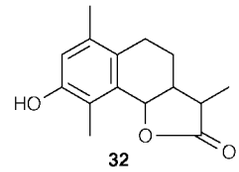

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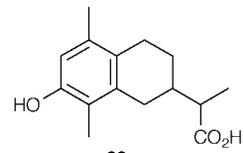

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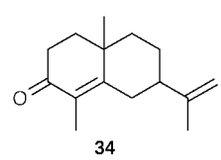
the unsaturated ketone followed from two further dehydrogenation experiments. Reaction of the ketone of tetrahydrocyperone with methylmagnesium iodide and dehydrogenation gave 1,2-dimethyl-7-isopropylnaphthalene. The extra methyl group located the position of the carbonyl group. The position of the methylene adjacent to the carbonyl and hence of the double bond, was established by condensation of α-cyperone to give a hydroxymethylene derivative. Reduction and dehydrogenation of this gave 1,3-dimethyl-7-isopropylnaphthalene. α-Cyperone was then synthesized from dihydrocarvone by a modification of the Robinson ring extension reaction.[42] The location of the unsaturated ketone in α-cyperone was one of the examples used by Woodward to illustrate the application of the correlations between structure and UV absorption that carry his name.[43]



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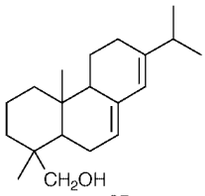
7 Studies on the di- and triterpenes in the 1920's and 1930's

Whilst the clarification of the structures of a number of the sesquiterpenoids was achieved during the 1920's and early 1930's, work on the di- and triterpenes took longer. Some of the evidence for the structure of abietic acid **21** reveals the strategies that were current. Although the underlying phenanthrene backbone of abietic acid had been established by the dehydrogenation to retene **22** in 1903, the location of the carboxy group and the double bonds took longer. A key piece of evidence involved reduction of the carboxylic acid to a primary alcohol, abietinol **35**. This was dehydrogenated to form a methylabietin, a homoretene which was eventually identified as 1-ethyl-7-isopropylphenanthrene **36**.[44] The formation of the ethyl group was rationalized in terms of a 1,2-shift of the methyl group to the carbinyl carbon atom. The C-1 substituent had been converted to an ethyl group and hence the methyl group and the carboxy group were attached to the same carbon atom. Other evidence for the position of the carboxy group and quaternary methyl group came from the energetic oxidation of abietic acid to two homologous tricarboxylic acids shown by further degradation to be **37** and **38**.[45] Vigorous oxidation to identifiable fragments was an important strategy which complemented dehydrogenation. These acids were also to play an important part later in the determination of the stereochemistry of the diterpenoids.[46] The location of the double bonds revealed some of the difficulties of working with natural products which readily isomerized. Since abietic acid gave a Diels–Alder adduct on heating with maleic anhydride, the double bond arrangement involving a homoannular diene was proposed. However this compound was also obtained from levopimaric acid. In the course of studies on dehydroabietic acid, a critical review was made[47] of the evidence for the position of the double bonds including a comparison of the UV



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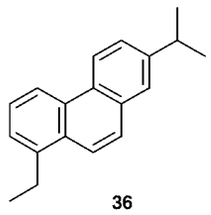

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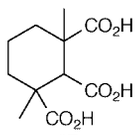

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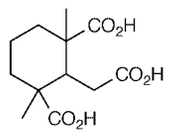

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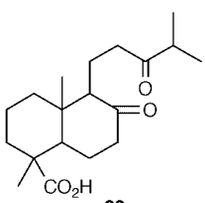
spectrum (λ_{max} 237.5 nm) with that of model compounds. This indicated the presence of a heteroannular diene. Definitive proof was obtained[48] by conversion of abietic acid to its iodotrihydroxy derivative. Hydrogenolysis of the iodine and oxidation product gave a diketo-acid **39**. Treatment of the latter with ammonia gave a dihydropyridine which was dehydrogenated to 8-azaretene **40**. This was then synthesized.[49] The position of the nitrogen atom reflected the position of the carbonyl groups in the diketo-acid and hence of the double bond in the parent natural product.



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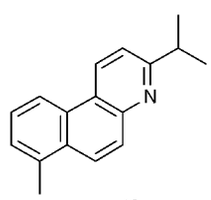

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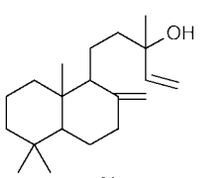
Manool **41** and manoyl oxide **42** were described[50] in 1934. Their underlying carbon skeleta were established[51] by the isolation of 1,2,5-trimethylnaphthalene and 1,2,8-trimethylphenanthrene on dehydrogenation. The position of the carbonyl group in ketomanoyl oxide **43** was established using a dehydrogenation strategy. Reaction with methylmagnesium iodide and dehydrogenation of the product gave 1,2,5,7-tetramethylnaphthalene and 1,2,6,8-tetramethylphenanthrene in which the position of the extra methyl group compared to manool and manoyl oxide, served to locate the carbonyl group.



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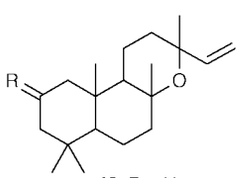
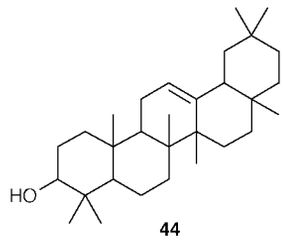


42 R = H_2
43 R = O

Although triterpenes had been isolated in the nineteenth century (the amyrins were isolated in 1839), there was considerable difficulty in establishing their molecular formulae. These were eventually established[52] by a combination of combustion analyses and the determination of acetyl and methoxy values for their esters. The hindered nature of several of their functional groups also limited progress. The stepwise reduction of a carboxy group *via* the acid chloride to the aldehyde and thence to a methyl group provided[53] a useful strategy for interrelating many triterpenes. The fortuitous presence of a double bond in ring C of the pentacyclic triterpenes (*e.g.* **44**) provided a site of reactivity. The vigorous thermal conditions of dehydrogenation reactions allowed retro-Diels–Alder reactions to occur and hence various alkylnaphthalenes such as 1,2,7-trimethylnaphthalene were obtained[54] from rings A and B or C and D.



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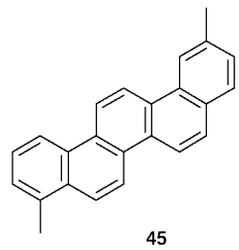

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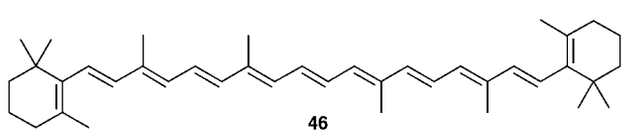
Significantly 1,8-dimethylpicene **45** was obtained, reflecting the pentacyclic skeleton of these triterpenes. The presence of an alkene on ring C also provided the opportunity for oxidative degradation and cleavage of the ring system.[55] Dehydrogenation of the resulting fragments led to the alkylnaphthalenes described previously.

8 The carotenoids, chromatography and UV spectroscopy

The isolation of a hydrocarbon pigment, carotene from carrots, was originally described by Wackenroder in 1831 and its identity with a pigment from green leaves was established by Willstätter in 1907.[56] The elucidation of the structure of the carotenoids in the 1930's revealed the impact of two major advances in technique. The first was the role of chromatography in the separation of closely related compounds including *cis*/*trans* isomers[57,58] and the second was the role of ultraviolet spectroscopy. Thus the chromatographic separation of 'carotene' into α-, β-, and γ-carotene was reported by Kuhn in 1932. There was particular interest in the relationship between the length of a polyene chain and the position of maximum absorption in the ultraviolet spectrum.[59] These correlations played an important part in establishing the length of the conjugated alkene chains. Ozonolysis of β-carotene **46** afforded α,α-dimethylsuccinic acid, α,α-dimethylglutaric acid and geronic acid **47**. Oxidation of the pigment with chromic acid gave six molecules of acetic acid per molecule indicating the substitution pattern of the double bonds.[60] The stepwise degradation of the carotenoids by the reaction with alkaline potassium permanganate and the formation of polyene aldehydes also played an important part in their structure elucidation.



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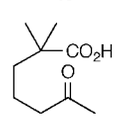

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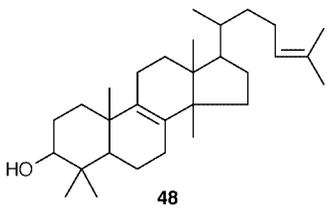
The existence of vitamin A as a fat-soluble growth factor was established in 1913 and its relationship to the plant carotenoids was noted by Steenbock. Its structure was established[61,62] in 1931 and confirmed by the synthesis of the perhydrovitamin from β-ionone in 1933.[63] The relationship between the carotenoids and vitamin A and the importance of vitamin A in vision was a significant synthetic challenge.[64]

The use of UV spectroscopy as a structural tool and in particular correlations between the position of maximum absorption and the substitution pattern of a chromophore such as a diene or an α,β-unsaturated ketone made a useful impact. The Woodward rules, described in 1941[43] for unsaturated ketones, have been particularly useful. Since it is possible to oxidize alkenes in the allylic position to afford an unsaturated ketone, this provided a strategy for locating double bonds. This was used, *inter alia*, in structural work in the triterpene series.[65] The changes in the ultraviolet spectrum associated with oxidation at the allylic positions (C-7 and C-11) of the tetrasubstituted

double bond of lanosterol **48** played an important role in the structure determination of this triterpenoid.



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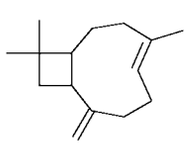
9 Sesquiterpenes with medium-sized rings

The classical methods of structure determination, in which dehydrogenation to aromatic hydrocarbons played a major role, were restricted to particular skeleta such as those based on a naphthalene or an azulene. The structure of the nine-membered ring of caryophyllene[49] was a matter of doubt for many years. Although caryophyllene had been known as a constituent of oil of cloves since 1834 and a crystalline nitrosite had been described[66] in 1892, its structure was not finally established until the 1950's. Oxidative degradation of caryophyllene **49** afforded three homologous cyclobutanedicarboxylic acids (*e.g.* **50**) which were synthesized in 1936[67] thus establishing the presence of the four membered ring. A suggestion[68] that caryophyllene contained a nine-membered ring was based on the infrared absorption of a nor-ketone derived from caryophyllene oxide. Definitive evidence for the structure of caryophyllene came from the base-catalysed cyclization of this nor-ketone (kobusone **51**) to a tricyclic hydroxyketone and its subsequent degradation.[69] This was accompanied by studies on the acid-catalysed cyclization products of caryophyllene and caryophyllene oxide which gave compounds possessing the caryolane and clovane skeleta. An X-ray crystal structure of one of the cyclization products, caryophanyl chloride, provided confirmatory evidence for the structure of caryophyllene.[70]



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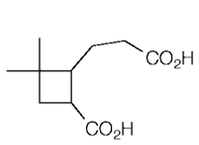

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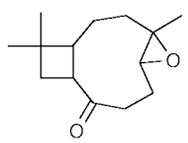

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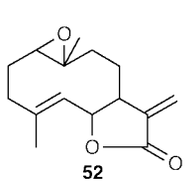
The propensity of many medium-sized rings to undergo cyclization reactions provided the basis for strategies for the elucidation of the structures of other families of terpenoid natural product. This is exemplified by the ten-membered ring of the germacranolide lactones such as pyrethrosin.[71] Acid catalysed cyclization of pyrethrosin **52** gave a decalin **53** which was correlated with ψ-santonin. However it was not until the advent of NMR spectroscopy that many other structures of this type were elucidated.



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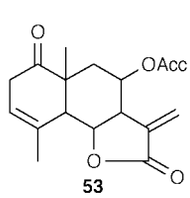

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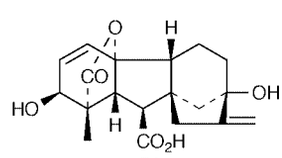
Whereas the existence of quaternary centres at ring junctions provided a stabilizing feature as far as chemical degradation was concerned leading to the identification of alkylcyclopentane and cyclohexane fragments, the presence of a quaternary methyl group often provided a block to establishing connectivities between protons by NMR spin decoupling studies. On the other hand the highly functionalized ten-membered rings of the sesquiterpene lactones readily lent themselves to this type of NMR study in which the contiguous relationship of proton–proton spin systems could be unravelled. Hence many of their structures were established in the 1960's.

10 The impact of IR spectroscopy

The elucidation of the structure of terpenes in the 1950's made use of classical dehydrogenation and oxidative degradation accompanied by interpretation of the IR spectra. The correlation between ring size and the frequency of the carbonyl absorption in the infrared spectrum played an important role in the identification of the ring size of ketones, lactones, and cyclic anhydrides.[72] This is exemplified by the characterization of the functional groups of the plant hormone, gibberellic acid **54**,[73] and by the studies on the diterpenoid fungal metabolite rosenonolactone **55** in which the presence of the γ-lactone (ν_{max} 1786 cm^{-1}) and the cyclohexanone (ν_{max} 1724 cm^{-1}) were established[74] by infrared spectroscopy.



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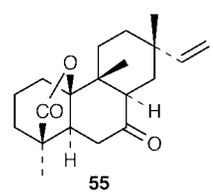

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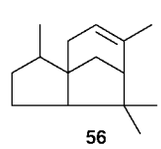
The correlation between ring size and absorption was particularly useful in establishing the size of bridged rings. This is exemplified by structural work on α-cedrene **56** in which the size of the ring containing the double bond was in doubt. Oxidative cleavage of this ring gave a norcedrene dicarboxylic acid **57**. This diacid was converted to an anhydride which had the infrared characteristics of a glutaric rather than a succinic anhydride.[75] This infrared study complemented the not always reliable Blanc method of determining the ring size of anhydrides in which pyrolysis of glutaric anhydrides gave cyclopentanones whilst succinic anhydrides did not react. The infrared absorption of the ring D ketones derived from the tetracyclic diterpenoids of the gibberellin and kaurene series characterized them as cyclopentanones.[73,76]



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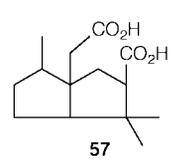

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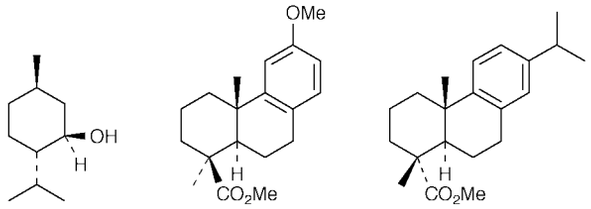
11 The determination of relative stereochemistry

The problems associated with establishing the relative stereochemistry of the terpenoids can be divided into those relating to the stereochemistry of the substituents and those associated with the stereochemistry of the ring junctions. Many of the classical pre-spectroscopic strategies yielded results in the 1940's and 1950's.

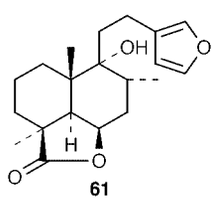
The assignment of the stereochemistry of the menthols **58** utilized[77] the comparative rates of their esterification with *p*-nitrobenzoyl chloride to determine the configuration of the alcohols at C-3. The two neomenthols were esterified more slowly than either menthol or isomenthol. This was attributed to steric hindrance by the isopropyl group and hence this group was placed *cis* to the hydroxy in the neomenthols. The much slower rate of hydrolysis of methyl podocarpate **59** compared to methyl dehydroabietate **60** was used[78] to reveal the greater steric hindrance in the former arising from interactions with the methyl group at C-10. The ester is an axial substituent in methyl podocarpate.



58 59 60

The *trans* A/B stereochemistry of the abietic and pimaric acids was established[46] by examining the p*K* values of the carboxylic acids **37** derived by oxidative degradation. These results were then extended to the triterpenes. The results of stereochemical studies in the terpene and steroid series provided many of the examples on which the theories of the conformational analysis of reactions were based.

The conformational stability of *trans* fused α-decalones was used to establish the B/C *trans* configuration in the pentacyclic triterpenes[79] and to establish the *trans* A/B fusion in the diterpenoids such as marrubiin **61**.[80] An interesting summary of the application of these reactions to the stereochemistry of the eudesmane group of sesquiterpenes appeared in 1960.[81]



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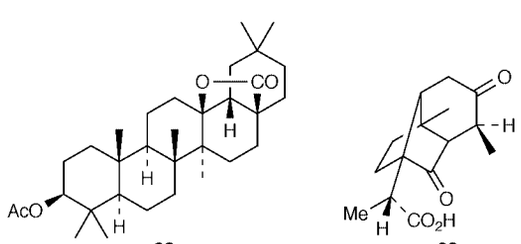
The strategy of using cyclization reactions such as lactonization reactions to establish the *cis* stereochemistry of the D/E ring junction in the pentacyclic triterpenes, in which the facile formation of the lactone **62** could only be accommodated with *cis* ring junction, is an example.[79,82]

The elucidation of the cyclization reactions of santonin to give the bridged santonic acid **63** played an interesting role in establishing the stereochemistry of santonin.[83] However the correct configuration at C-11 was eventually established by X-ray analyses of 2-bromo-α-santonin and 2-bromo-β-santonin.[84]

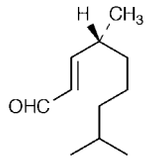


62 63

12 The determination of absolute stereochemistry

A number of monoterpenes were known to occur in both enantiomeric forms. Optically active methylsuccinic and β-methyladipic acids had been obtained as degradation products and these were correlated with D-(+)-glyceraldehyde. Other interrelationships between the monoterpenoids provided useful stereochemical correlations.[85] Hence when the absolute stereochemistry of sodium rubidium tartrate was established by X-ray crystallography and the correlation made with D-(+)-glyceraldehyde,[86] the absolute stereochemistry of many of the monoterpenes was established.

Synthetic interrelationships between carvone and the sesquiterpenes of the eudesmane series and between eudesmol and the steroid series led to a correlation of their absolute stereochemistry.[87] An important degradation of cholest-14-en-3β-ol to give a fragment **64** containing the steroid side chain and



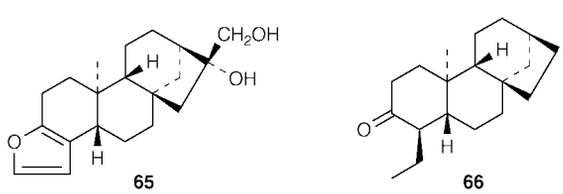
64

which was related to (+)-citronellal, established[88] the absolute stereochemistry of the steroids at C-20.

The extension of the method of molecular rotation differences, which had been successfully applied to correlations of stereochemistry in the steroid series, to similar changes in the triterpenes[89] and then to the sesqui- and diterpenes,[90] provided useful correlations of absolute stereochemistry.

13 Optical rotatory dispersion and circular dichroism

The closely related methods of optical rotatory dispersion and circular dichroism played an important role in establishing the absolute stereochemistry of terpenoid natural products.[91] Although some of the first observations of the change of optical rotation with wavelength were made with camphor, the principal applications to stereochemistry did not begin until the 1950's with the work of Djerassi and Klyne. The octant rule, for predicting the sign of the Cotton effect in the ORD curve of ketones in a chiral environment, was based on the position of substituents adjacent to the ketone.[92] This development meant that it was possible to assign the absolute stereochemistry to a number of terpenoids. A particular impact of these studies was the demonstration that a number of diterpenoids belonged to the antipodal series, *i.e.* their absolute stereochemistry at the A/B ring junction was opposite to that of the steroids and other diterpenoids such as abietic acid. For example the diterpenoid cafestol **65** was converted into the ethylketone **66**.[93] The ORD curve of this ketone was the mirror image of the curve from 4α-ethylcholestan-3-one and hence the absolute stereochemistry of the A/B ring junction of cafestol is opposite to that of the steroids. Studies on the ring D ketones of a number of diterpenoids were important in establishing their absolute stereochemistry.[76,94]



65 66

14 The absolute stereochemistry of terpenoid secondary alcohols

A number of methods have been developed for establishing the absolute stereochemistry of a secondary alcohol. Studies on the addition of Grignard reagents to the α-keto-esters of optically active terpenoid alcohols showed[95] that asymmetric induction could be used to establish the stereochemistry of a chiral alcohol. The chirality of the atrolactic acid that was formed reflected that of the original secondary alcohol. Horeau's method[96] was based on the kinetic resolution by the alcohol in its esterification by 2-phenylbutyric anhydride. The method is based on using a racemic mixture of the anhydride. One enantiomer will react faster with the chiral secondary alcohol than the other. The optical activity of the remaining 2-phenylbutyric acid will thus reflect the chirality of the secondary alcohol.

An NMR method based on derivatization with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) has become a popular strategy for establishing the absolute stereochemistry of terpenoid alcohols.[97] Although it was originally based on an analysis of the ^{19}F NMR spectrum, a more reliable

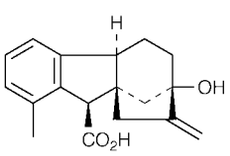
version uses the high field ^{1}H NMR spectrum. A correlation was established between the absolute stereochemistry of terpenoid secondary alcohols and the $\Delta\delta$ ($\delta_S - \delta_R$) values of adjacent protons for the (*R*)- and (*S*)-MPTA esters. This correlation was successfully applied in establishing the absolute stereochemistry of a number of marine terpenoids of the cembranolide and xenicane series.[98]

In a few instances the absolute configuration of a terpenoid has been determined by X-ray crystallography of a heavy atom derivative, *e.g.* an ester or an amide containing a bromine atom.

The assumption is often made that all of a group of terpenes that occur in a particular plant belong to the same enantiomeric series. However there have been a number of reports where, for example, enantiomeric labdane diterpenes co-occur in the wood of *Oxystigma oxyphyllum*[99] and in the leaves of *Mimosa hostilis*.[100] Hence this assumption in terpenoid structure elucidaton is not always valid.

15 The transition to spectroscopy driven strategies

The elucidation of the structure of gibberellic acid **54** represents the transition between the strategies that were dominated by chemical degradation and those that were driven by the use of physical methods. The work in the 1950's concentrated on establishing the structure of the acid-catalysed degradation and rearrangement products, allogibberic **67** and gibberic **68** acids.[73] These studies utilized classical dehydrogenation reactions to form substituted fluorenes which were then synthesized, and secondly stepwise degradative sequences in which rings C and D were cleaved.[101] This chemical work was supplemented by information drawn from ultraviolet and infrared spectroscopy. However several aspects of the structure of gibberellic acid itself which were described in 1958, utilized assignments of the ^{1}H NMR spectrum.[102] The full structure and stereochemistry was based on a combination of chemical and spectroscopic studies in which ^{1}H NMR spectroscopy played an important role in interrelating protons, for example the *trans* relationship between H-5 and H-6.[103] Independent X-ray crystallographic studies of heavy atom derivatives were also published.[104] More recent studies on the structures of novel gibberellins are entirely determined by physical methods, particularly mass spectrometry.[105]



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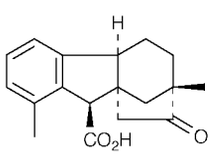

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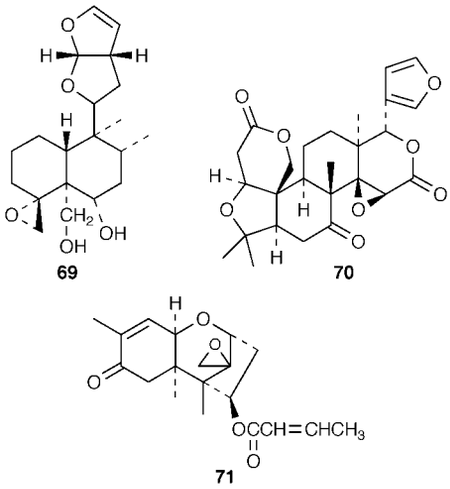
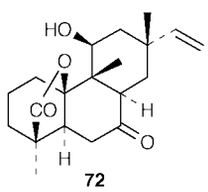
Spectroscopic methods played a significant role in the final elucidation of the structures of a number of highly oxygenated terpenoid bitter principles including clerodin **69**[106] and limonin **70**.[107] In these cases prior chemical work had established partial structures but the evidence for the full structure hinged on the ability of ^{1}H NMR spectroscopy to link groups of atoms. In the sesquiterpene area the elucidation and revision of the structure of the trichothecenes (*e.g.* **71**)[108] revealed the ability of ^{1}H NMR spin decoupling studies to cope with highly oxygenated compounds, thus avoiding the need for the stepwise removal of functional groups and the structural simplification which characterized the earlier chemically driven strategies.

The increasing role of X-ray crystallography in structure determination was also illustrated at this time. A number of structures, such as those of clerodin[109] and limonin,[110] were also determined by crystallographic methods in 'competition' with the classical chemical and spectroscopic studies. Although the theory of non-heavy atom structure determination was known, the majority of the terpenoid structures that were reported in the 1960's were based on heavy atom derivatives. One of the earliest terpenoid non-heavy atom structures to be reported[111] was that of rosein III (11β-hydroxyrosenonolactone) **72** in 1970.





16 The impact of instrumental chromatographic techniques

The development of instrumental chromatographic techniques for the separation of natural products, particularly terpenoids, had widespread ramifications in structure elucidation.[112] The gas chromatographic analysis of essential oils had revealed their complexity and when coupled with mass spectrometry led to the elucidation of the structures of a number of new mono- and sesquiterpenoids. The understanding of factors which affect the fragmentation patterns of terpenoids in the mass spectrometer and the use of these as structural tools has become very important. The sensitivity of the methods gave a stimulus to insect chemistry in which a number of volatile mono- and sesquiterpenoids play an important role as pheromones.[113] The conversion of the gibberellin plant hormones to their esters and trimethylsilyl ethers followed by their separation and identification by gas chromatography–mass spectrometry led not only to the elucidation of the structures of over 120 of these compounds but also to their quantitation at various stages of plant development.[105,114] Confirmatory synthetic and model studies have become a very important part of these instrumental strategies in which very small amounts of the natural product are examined.

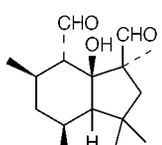
The development of HPLC as a separation tool and the link with spectroscopic methods has had a similar impact on the isolation and structure elucidation of more polar terpenoid natural products, particularly compounds from marine sources.

17 The impact of biosynthetic studies

The development of plausible biogenetic schemes based on the isoprene rule, the cyclization of polyprenyl chains and the rearrangement of carbocationic intermediates, provided the means of evaluating terpenoid structures.[115] The discovery of the role of mevalonic acid in the biosynthesis of cholesterol[116] and the subsequent demonstration of its incorporation *in vivo* into terpenoid fungal metabolites led[117] to studies which changed this biogenetic speculation into biosynthetic evidence.

Studies in terpenoid biosynthesis may be divided into four phases. The first phase involves the origin of the isoprene unit,

isopentenyl pyrophosphate. This is undergoing a resurgence of interest as a consequence of the discovery of the non-mevalonate deoxyxylulose pathway to terpenoid compounds.[118] The second stage involves the stepwise polymerization of the isoprene units to form the acyclic polyprenyl precursors of the terpenoids such as geranyl, farnesyl and geranylgeranyl pyrophosphate. The third stage involves the cyclization of these to form the underlying carbon skeleta of the various families of terpenes and the final stage involves establishing the sequence and stereochemistry of the various hydroxylations and oxidations which lead to the individual families of terpenoid natural product. Whilst it is not the purpose of this review to give an account of the development of terpenoid biosynthesis, it is important to note that these studies have had a significant impact on terpenoid structure elucidation. Implicit in the sequences of the later stages of many terpenoid biosyntheses are a number of 'missing' links. This has provided the stimulus for the careful examination of extracts in order to identify the relevant compounds, the structural characteristics of which could be predicted from the biogenetic speculation. The study of the biosynthesis of the gibberellin plant hormones[105] and of many sesquiterpenoid fungal metabolites, such as those (*e.g.* **73**) of *Botrytis cinerea*,[119] provide examples of this. The need to prepare labelled compounds has also led to a reinvestigation of various aspects of degradative chemistry. The use of carbon-13 labelled precursors has provided the stimulus for the assignment of the carbon-13 spectra of terpenoid natural products whilst the development of specialized biosynthetic techniques such as the use of inhibitors and of plant tissue culture has led to the isolation of novel terpenoid natural products.



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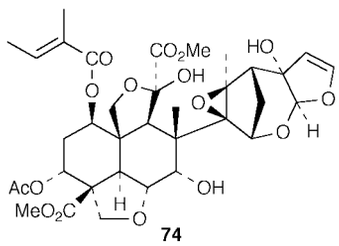
An understanding of the pathways can lead to a rationalization of the role of various functional groups in a terpenoid structure. Thus a hydroxy group or a double bond may be the consequence of a cyclization step, it may be the structural code for a particular biosynthetic sequence or it may be part of the 'dumping' or 'control' mechanism of biosynthesis.

18 The role of NMR spectroscopy

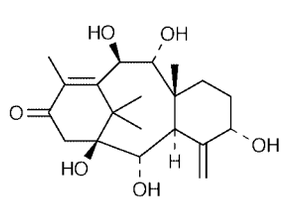
Very early in the application of NMR spectroscopy to structural problems, it became apparent that changes in the chemical shift and multiplicity of signals consequent upon chemical changes provided useful methods of assigning signals and interrelating protons. Since many terpenoid natural products occur in plants in groups differing only in the number and position of hydroxy groups or esters, the changes in spectra associated with changes in functionality provided the means of establishing the structures of groups of natural product. Thus there was a change in the 1960's from investigations which concentrated on the structure of one natural product to those which elucidated the structures of a number of related compounds. This change of strategy is exemplified by the studies on the sesquiterpenoid lactones. It facilitated a number of phytochemical surveys exemplified by the work on the Compositae and the Labiatae and when coupled with UV spectroscopy, on the work on the pigments of the *Coleus* and *Plectranthus* species.[120] The recognition of characteristic features in the NMR spectra of terpenoid natural products facilitated the identification of the many clerodanes found in the Labiatae, some of which are insect antifeedants.[121] The branched chain nature of the isoprene unit leading to the generation of a quaternary methyl group by typical terpenoid cyclizations and furan rings from the terminal isoprene units were helpful in this context.

The application of Fourier transform methods to the recording of ^{13}C NMR spectra meant that useful data could be collected for a range of terpenoids during the early 1970's and then applied to structural problems. One of the earliest examples of this was in the structure elucidation of azadirachtin **74**.[122] The changes in the ^{13}C NMR spectrum arising from the insertion of a hydroxy group made this a particularly useful tool in the study of groups of terpenoid natural products.



74

The structural studies on the taxanes illustrate the impact of NMR strategies on structure elucidation. Studies reported between 1958 and 1962 on taxicin 1 **75** and *O*-cinnamoyltaxine followed[123] a classical degradative pattern in which the two vicinal diols were cleaved by periodate to give an enolic and a neutral component. The structures of these were determined by predominantly chemical means although examination of the ^{1}H NMR spectrum led to a correction of the structure of the neutral component.[124] However the elucidation of the complete structure of taxane carbon skeleton in 1966 involved the assignment of the ^{1}H NMR spectrum.[125] The importance of the tumour inhibitory activity of Taxol® **76** and the shortage of material meant that X-ray crystallography was the method of choice for its structure elucidation in 1971.[126] Nevertheless heavy atom derivatives had to be used and the structure was obtained on two parts of the molecule. The way in which these parts were assembled followed from the ^{1}H NMR spectrum. Advances in NMR spectroscopy and particularly the use of two dimensional NOESY spectra, meant that in the 1990's it was possible to define the solution conformation of Taxol® and Taxotere®.[127] The search for novel taxanes from *Taxus* species and their structure elucidation has relied to a major extent on NMR methods.



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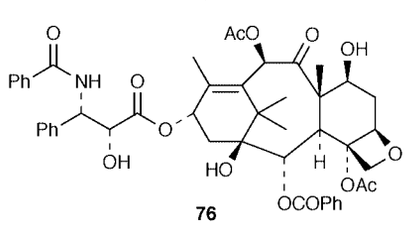
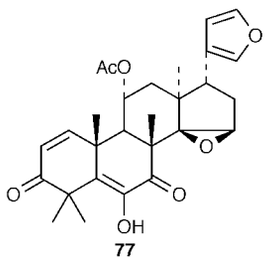


76

There are a series of highly oxidized nortriterpenes which have been obtained from the heartwood of a number of members of the Meliaceae, particularly trees of the genera *Cedrela* and *Khaya*. Once the basic NMR characteristics of these compounds were established, the elucidation of the structures (*e.g.* **77**) of a number followed relatively quickly.[128] Another series of complex terpenoid structures in which NMR spectroscopy played an important role were the

naturally occurring tumour promoting phorbol esters exemplified by resiniferatoxin **78**.[129]



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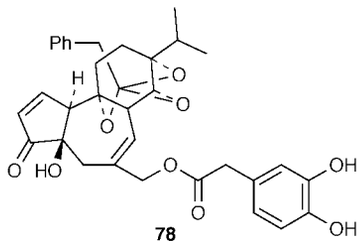
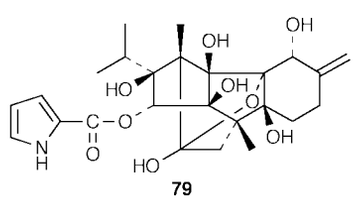


78

The development of chloroquine resistant strains of the malaria parasite, *Plasmodium falciparum*, led to the search for novel antimalarials and to the isolation of an unusual sesquiterpenoid cyclic peroxyketal (qinghaosu) from *Artemisia annua*. The structures of this compound and its relatives were established by a combination of X-ray crystallography and NMR methods.[130]

19 Strategies based on 2D NMR correlations

In the mid 1980's strategies based on two dimensional NMR correlations began to be developed. These included two dimensional ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra. An early application of 2D ^{13}C–^{1}H spectroscopy may be found in the assignment of the structure of an insecticidal diterpenoid, 9,21-didehydroryanodine **79** from *Ryania speciosa*.[131] Another example of two dimensional long range ^{13}C–^{1}H correlations was their use in the interrelationship of the ^{1}H signals from the methyl groups of esters of 19-hydroxyingol esters **80** from *Euphorbia poisonii*.[132]



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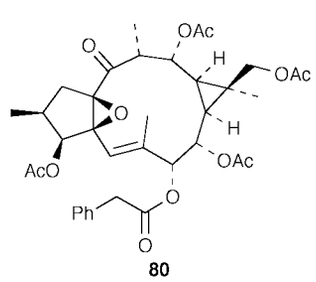
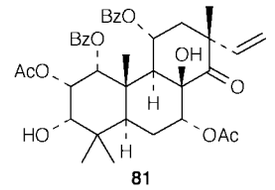


80

Whilst these methods led to the recognition of specific arrangements of atoms with characteristic NMR chemical shifts, multiplicities and integrals, the application of two-dimensional NMR methods[133] to the assembly of fragments involved the development of heteronuclear multiple bond correlations (HMBC). A typical strategy using 2D NMR data, is to link the ^{1}H and ^{13}C resonances *via* a heteroCOSY experiment and then to establish the ^{1}H–^{1}H correlations by a homonuclear COSY experiment. From these correlations it is then possible to infer the C–C connectivities and to begin to establish part structures. When the part structures are terminated by a quaternary centre, it may then be possible to establish long range connectivities using an HMBC spectrum. A planar structure may be derived this way. The relative stereochemistry may then be ascertained by 2D nuclear Overhauser exchange spectroscopy (NOESY) or by the measurement of proton coupling constants. The absolute stereochemistry may be assigned either by chiroptical methods or by using an NMR method based on derivatization with a chiral reagent. Over the last ten



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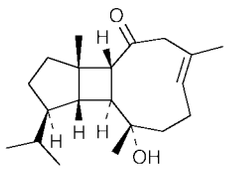

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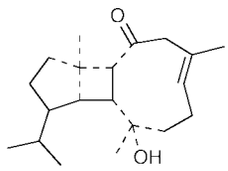

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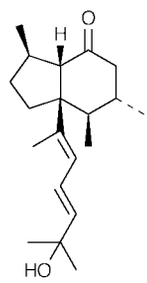

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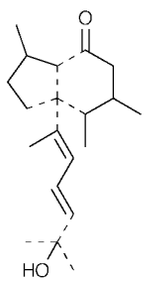
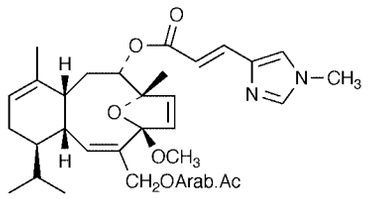


85

years there have been very many examples of this strategy using two dimensional techniques particularly in the elucidation of the structures of diterpenoids from marine sources and in the search for novel taxanes.

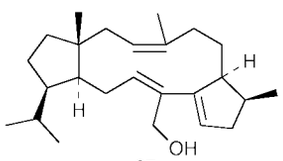
One example is the elucidation of the structure of the orthosiphols A **81** and B which are diterpenoid antiinflammatory agents from the medicinal herb *Orthosiphon stamineus*.[134] The planar structure was identified by the use of ^{1}H–^{1}H and ^{13}C–^{1}H COSY correlations and the relative stereochemistry from the NOESY spectrum. The chirality was established by the exciton chirality method based on the interaction between the benzoate groups. There are also many examples of this strategy in the studies on the chemistry of marine organisms. One example is that of sarcoglane **82**, a cytotoxic diterpene obtained from the coral *Sarcophyton glaucum*.[135] The EIMS, IR and one dimensional NMR spectra revealed the nature of the functionality whilst 2D NMR spectra (COSY) suggested the presence of the two substantial fragments shown in **83**. The connectivities between these partial structures were established by the C–H correlations in the HMBC spectrum to give the planar structure. The relative stereochemistry and in particular the geometry of the ring junctions was assigned on the basis of NOE experiments. The liverworts have been the source of many interesting terpenoids. In the structure of hatcherone **84** from a liverwort, *Barbophozia hatcheri*, the ^{1}H–^{1}H and ^{1}H detected heteronuclear multiple quantum coherence (HMQC) spectrum established[136] the presence of the units shown in **85**. The connectivity of these units was clarified by the HMBC spectrum and the relative stereochemistry was established by nuclear Overhauser spectroscopy. The branched chain nature of the isoprene unit lends itself to these studies. Thus in these examples the angular methyl groups lying within the heart of the molecule possess useful transannular interactions. The absolute stereochemistry of hatcherone was established by the application of the octant rule to the sign of the Cotton effect in the CD spectrum.

When another marine diterpenoid natural product, eleutherobin **86**, with a similar activity to that of Taxol but with a completely different structure, was discovered[137] in 1997, the structure elucidation was completely dominated by the use of two dimensional homo- and heteronuclear correlation spectra. The structure determination of many marine natural products follows this strategy.



86

These methods are not constrained by ring size and may cope with a diversity of skeletal types. An illustration of this involves the elucidation of the structure of a sesterterpenoid, nitiol **87**,[138] which was isolated from a Peruvian folk medicine, hercampuri (*Gentianella nitida*). This terpenoid contains a twelve-membered ring. Interpretation of the ^{1}H–^{1}H COSY and HMQC spectra led to the identification of the part structures shown in **88**. The HMBC spectrum revealed the connectivity of these partial structures illustrating the way in which the HMBC spectrum can bridge quaternary centres. The relative stereochemistry followed from the NOESY spectrum.



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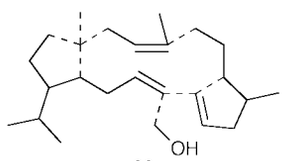

88

However reviewing strategies of this type sometimes leaves an impression of a structure that is consistent with the data but not necessarily proven by it to the exclusion of other possibilities. These approaches may be linked to computer assisted structure elucidation programs[139] which have as their objective the generation of all possible structures that are consistent with the spectroscopic data, minimizing the impact of human prejudice favouring particular structures but at the same time taking away the intellectual challenge posed by this aspect of natural product chemistry.

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