Leopold Ružička

Multimembered rings, higher terpene compounds and male sex hormones

Nobel Lecture, December 12, 1945

This lecture should have been delivered in December 1939*, but owing to a postponement of 6 years forced by the intervention of war, I have been able to use some results obtained in the meantime to round off the overall picture. Consequently I can now report on the results of 25 years of laboratory work in two fields of the chemistry of alicyclic compounds: multimembered rings and higher terpene compounds (polyterpenes), to which the third group named in the title, the male sex hormones, is closely related.

Alicyclic compounds scarcely differ in their chemical properties from their aliphatic analogues. Many investigations, especially during the past twenty-five years, have shown, however, that in spite of this close chemical similarity aliphatic compounds and their alicyclic analogues can exhibit entirely different physiological properties. We see that certain well-defined physiological properties may appear or disappear in the transition from an aliphatic to an alicyclic compound or vice versa. As a result of the accumulation of such observations, alicyclic compounds to which only scant biochemical importance was attached as little as 20-25 years ago, have now advanced to the forefront of interest.

Multimembered rings

After Kekulé, in 1865, first introduced the carbon ring into structural chemistry in his formula for benzene, the 6-membered ring maintained its unique position in the taxonomy of organic chemistry for several decades. Indeed, a heterocyclic 5-membered ring containing nitrogen became known in 1870 as a result of Adolf von Baeyer's work on indigo; but this did not imme-
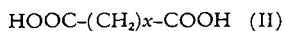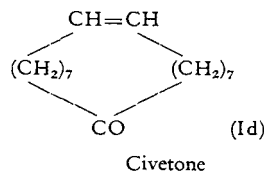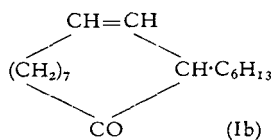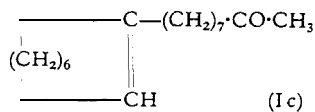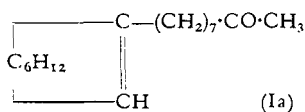
* Data available at that time formed the subject of a number of lectures which I delivered between December 1939 and April 1940 in Zurich, Zagreb, Utrecht, Amsterdam, Delft, Liége, Brussels and Paris. This material was published in Croat in the Zagreb medical journal *Lijecnicki vjesnik,* Vol. 62, July 1940.

diately stimulate attempts to prepare small carbocyclic rings. In the first Peeler Lecture delivered before the Chemical Society in London in 1929, W. H. Perkin Jr.[1] described "the early history of the synthesis of closed carbon chains". He dealt not only with the reactions which led to the synthesis of small carbon rings during the period 1881-1883, but also with the pessimistic views of the leading chemists of that time regarding the possible existence of carbocycles other than the 5- and 6-membered rings. Adolf von Baeyer, Emil Fischer, and Viktor Meyer advised Perkin not to waste his time on attempts to prepare such compounds, which could "hardly be capable of existence".

The systematic researches of Perkin and the roughly contemporary work of other authors - special mention should be made of Markovnikov[2] and Freund[3] - led, in the course of a few years, to the establishment of the chemistry of small, i.e. 3-to 5-membered carbon rings. The 7-and the 8-membered ring was also subsequently prepared by various methods, whilst attempts by Zelinsky and Willstätter to prepare the 9-membered ring were unsuccessful, and accordingly the prejudice regarding the impossibility of synthesizing carbocycles with more than 8 members in a ring continued. The unsuccessful attempts to expand the cyclic system beyond the 8-membered ring led to the view that von Bayer's strain theory, which was based on the work of Perkin and others interpreting the ease of formation and the properties of 3- to 6-membered rings, is also applicable to ring systems containing more than six members. According to this theory, the inaccessibility of larger rings is due to the strain resulting from the arrangement of all the ring members in one plane, and the consequent deviation of the valency angles from their normal position.

Sachse[4] had already pointed out that strain free models are possible for rings containing 6 and more members, provided that Baeyer's assumption of a planar structure is disregarded. Böeseken[5] showed experimentally in 1922-1924, that a planar structure is improbable for 6- and 7-membered rings, and in 1926 Hückel[6] proved chemically - as far as such a proof can be achieved by chemical means - that the 6-membered ring is not planar, at least in the case of decalin.

At that time, from 1922 on, I was engaged in the elucidation of the constitution of the natural musk perfumes, civetone $C_{17}H_{30}O$, and muscone $C_{16}H_{30}O$, and was able to show conclusively that civetone contains a 17-membered, and muscone a 15-membered carbon ring. In the original experiments, which were carried out on civetone[7], I was hindered less by the caprices of the substance itself than by the general prejudice, shared by my-

$$\begin{array}{c} \text{C---(CH}_2)_7\cdot\text{CO}\cdot\text{CH}_3 \\ \text{C}_6\text{H}_{12} \parallel \\ \text{CH} \qquad (Ia) \end{array}$$

$$\begin{array}{c} \text{C---(CH}_2)_7\cdot\text{CO}\cdot\text{CH}_3 \\ (\text{CH}_2)_6 \parallel \\ \text{CH} \qquad (Ic) \end{array}$$

$$\begin{array}{c} \text{CH=CH} \\ (\text{CH}_2)_7 \qquad \text{CH}\cdot\text{C}_6\text{H}_{13} \\ \text{CO} \qquad (Ib) \end{array}$$

$$\begin{array}{c} \text{CH=CH} \\ (\text{CH}_2)_7 \qquad (\text{CH}_2)_7 \\ \text{CO} \qquad (Id) \\ \text{Civetone} \end{array}$$

$$\text{HOOC--(CH}_2)x\text{--COOH} \quad (II)$$

$x = 7$, Azelaic acid $x = 5$, Pimelic acid
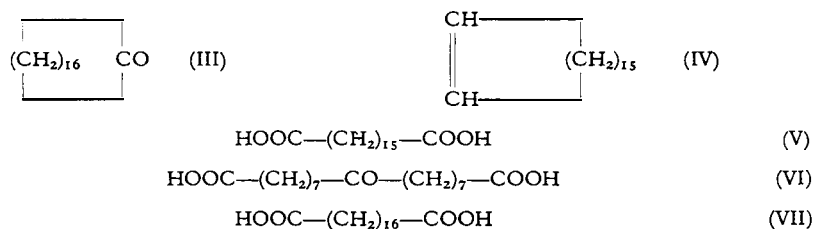$x = 6$, Suberic acid $x = 4$, Adipic acid

self, against the probability of the existence of a 15- and a 17-membered ring.

Degradation of civetone with a large quantity of potassium permanganate or with ozone, followed by further oxidation of the degradation products with hypobromite, yielded a mixture of normal dicarboxylic acids (general formula II), which could be readily separated by fractional distillation of the esters. The pure acidic products were characterized as azelaic, suberic, pimelic, adipic and succinic acids.

Formula (Ia) was therefore considered as a possible partial formula for civetone*, on the assumption that it contained a substituted 6-membered ring. An analogous formula with the grouping $(CH_2)_7$ in the ring seemed less likely since this represents a 9-membered ring. Formulae of the type (Ib) involving a ring with at least 11 members in which both the keto-group and the double bond lie in the ring, were even less likely. Furthermore, oxidation of civetone with a small amount of potassium permanganate or with ozone yielded a keto dicarboxylic acid $C_{16}H_{28}O_5$ or $C_{17}H_{30}O_5$, which on further degradation with potassium permanganate and alkaline hypobromite gave only some of the above-mentioned normal dicarboxylic acids (II). Since there was no indication of the formation of degradation products other than the normal dicarboxylic acids, a normal chain structure (IC containing an 8-membered ring) had also to be considered for the portion of the carbon skeleton denoted by C_6H_{12} in formula (Ia).

* In. the original publications, no mention was made of the fact that, in the course of the work, the supposed formulation for civetone did not progress in one step from the 6-membered to the 17-membered ring. I believe, however, that the subsequent description of some considerations involved in the elucidation of the constitution of civetone may be of some interest.

Finally, when we found that both the hydrogenation product of civetone, i.e. dihydrocivetone (III), and the unsaturated hydrocarbon (IV) obtained by Wolff-Kishner reduction of civetone give the same dicarboxylic acid $C_{17}H_{32}O_4$, were we forced to discard formulae of the type (Ia)-(1c) and to consider the completely symmetrical formula Id for civetone. The formulation of the dicarboxylic acid $C_{17}H_{32}O_4$ as heptadecane-1,17-dioic acid (V), as suggested by these considerations, was irrefutably confirmed by comparison with the synthesized product[8]. The carefully purified keto dicarboxylic acid $C_{17}H_{30}O_5$ was identified as 9-ketoheptadecane-1, 17-dioic acid (VI), since it could be synthesized by heating a monoester of azelaic acid with iron. A further confirmation of the presence of the 17-membered ring in civetone is provided by the synthesis of dihydrocivetone by cyclisation of the synthetic octadecane-1, 18-dioic acid (VII).

$$(CH_2)_{16} \quad CO \qquad (III) \qquad\qquad \begin{matrix} CH \\ \| \\ CH \end{matrix} \quad (CH_2)_{15} \qquad (IV)$$

$$HOOC—(CH_2)_{15}—COOH \qquad\qquad (V)$$

$$HOOC—(CH_2)_7—CO—(CH_2)_7—COOH \qquad\qquad (VI)$$

$$HOOC—(CH_2)_{16}—COOH \qquad\qquad (VII)$$

The fact that dihydrocivetone still possesses practically the same odour as civetone suggested that in the case of muscone ($C_{16}H_{30}O$), which does not contain a double bond, the musk odour is also due to the presence of a multi-membered ring. Since muscone is optically active, it could contain at most a 15-membered ring with a methyl side-chain, if the above assumption is valid. This conclusion was found to be correct, and it was shown that muscone is the optically active β-methylcyclopentadecanone (VIII).

$$CH_3—CH \underset{(CH_2)_{12}}{\overset{CH_2}{<\quad>}} CO \qquad (VIII)$$
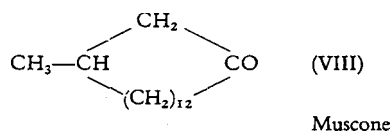
Muscone

Civetone(Id) and muscone (VIII) seemed, both as natural products and in the system of organic chemistry to break the old empirical rule "Natura non fecit saltus". Speculations on the probable mechanism of the biochemical formation of these two ketones lead to a plausible explanation for their unusual

structure. Civetone could perhaps originate in the civet cat by ω-oxidation of oleic acid (IX), followed by cyclization of the unsaturated dicarboxylic acid (X). Muscone could also be a product of fatty acid metabolism. Thus the constitution of the two animal musk perfumes may perhaps be the result of cyclization reactions at the end of the long chain of higher fatty acids[9].

$$CH-(CH_3)_7-CH_3 \atop \| \atop CH-(CH_2)_7-COOH \quad (IX) \qquad CH-(CH_)_-COOH \atop \| \atop CH-(CH_2)_7-COOH \quad (X)$$

The 15- and 17-membered rings were unable to maintain for long their initially lonely position in the system of organic chemistry. The well-known method of preparing cyclic ketones by the dry distillation of Ca-salts of dicarboxylic acids could be used after slight modification, to prepare the entire homologous series of cyclic ketones, starting with the 9-membered ring and extending beyond the 30-membered ring. Systematic experiments showed that salts of dicarboxylic acids with metals of groups 3 and 4 of the periodic system, and in particular with cerium or thorium, are suitable for this purpose[10]. In all cases examined, it was possible to isolate the expected cyclic ketone. Whereas the yield falls continuously from the 6-membered ring (approx. 70%) to the 10-membered ring (approx. 0.1%), it begins to rise once more with the 11-membered ring, and reaches a maximum (about 5%) with the 16-membered ring, after which it again falls to a few per cent. The constitutions of all the cyclic ketones prepared were determined by oxidation to the normal dicarboxylic acids with the same number of carbon atoms.

The accessibility of some ring systems is facilitated by the fact that the formation of the anticipated monoketones in the cyclisation is accompanied by the formation of the cyclic diketones (XI) with molecules twice as large. This method gave the 34-membered diketone, formed together with dihydrocivetone, as the largest carbocyclic system. This could be reduced to the 34-membered monoketone and the corresponding hydrocarbon,

$$\begin{array}{c} \overline{}-CO-\overline{} \\ (CH_2)_x \qquad\qquad (CH_2)_x \qquad (XI) \\ \overline{}-CO-\overline{} \end{array}$$
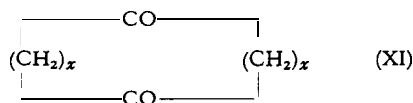
The derivatives of the 30-membered ring in particular became relatively readily accessible for investigation, since cyclotriacontane-1,16-dione is obtained as a by-product in the technical preparation of cyclopentadecanone («Exaltone»), which is used as a synthetic musk perfume.

Table I.

Number of units in the polymethylene	2	3	4	5	6–8	15	30
Heat of combustion in kcal per CH_2-group	170	168	165	159	158	157	156

The low yields of the higher ketones are not due to any chemical instability comparable to that of the 3-to 4-membered ring compounds, since they can be heated to 400° or with concentrated hydrochloric acid in a sealed tube, to 150° without undergoing ring cleavage or isomerisation. The heats of combustion of a number of multimembered ketones and hydrocarbons were determined as a physical evaluation of their stabilities. Table I shows the heats of combustion per CH_2-group in the 8-, 15-, 17- and 30-membered polymethylenes[11] as well as the known values for ethylene and the 3- to 8-membered polymethylenes. It can be clearly seen that, as the number of members of the ring increases beyond six, the heat of combustion does not increase; on the contrary it exhibits a slight but definite decrease, and with the 30-membered ring, reaches the value of about 156 kcal characteristic for an aliphatic CH_2 group.

The spatial molecular structures of a number of multimembered ring compounds were studied by X-ray methods[12]. The measurements on the higher-molecular polymethylenes clearly show an approximately linear increase in the longest period with the number of carbon atoms in the molecule. Thus for instance, from the difference in length between the 24-membered and the z&membered cyclic diketones it was possible to calculate the value of I.25 Å for the length of a chain unit and a value of I8.7 $\cdot 10^{-16}$ cm^2 for the half cross-section of the molecule, i.e. the cross section of the single CH_2-chain. These values closely agree with the corresponding figures for normal paraffin hydrocarbons. Thus the structure of the multimembered ring compounds appears to consist essentially of two parallel chains of CH_2 groups closed at both ends. For purposes of comparison, we show here photographs of the 6-, 10-, and 15-membered hydrocarbons (cyclohexane, cyclodecane and cyclopentadecane) as represented by the well-known Stuart models for carbon and hydrogen (cf. Figs. 1-3). In the case of the 6-membered ring, the inner portion of the molecule consists entirely of carbon atoms, whilst the hydrogen atoms form an outer shell. As the number of ring members increases, some of the hydrogen atoms come to lie inside the ring, and the two parallel chains, corresponding to the model of normal paraffin chains, are clearly visible in the I s-membered ring, and even more so in the higher homologues.
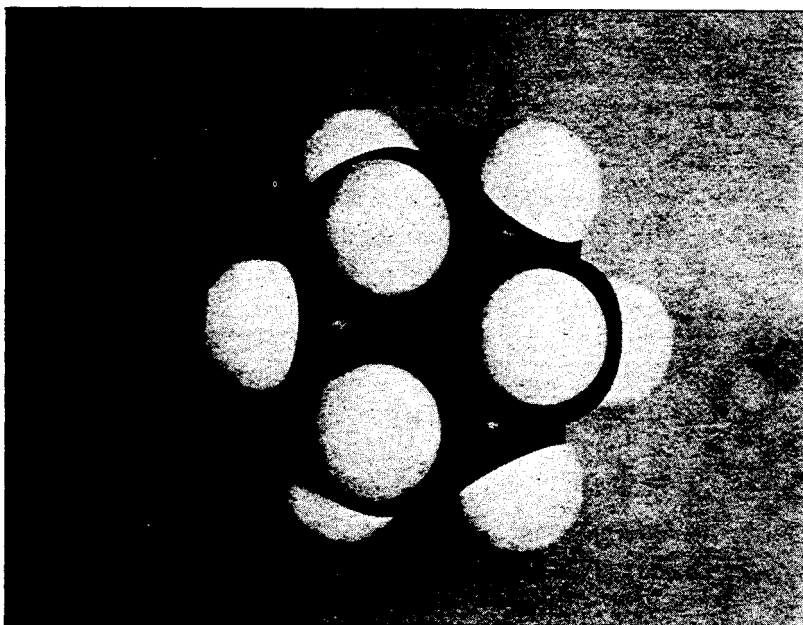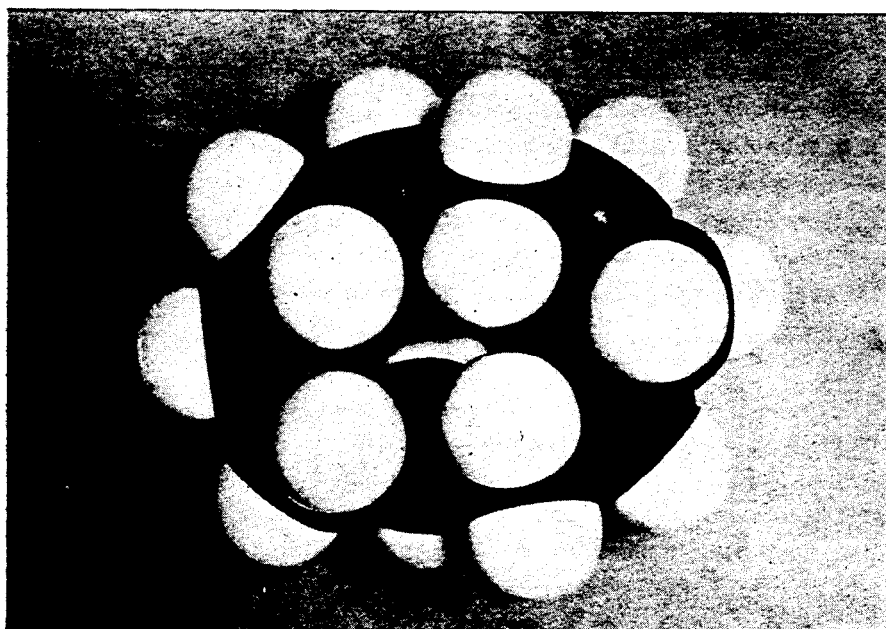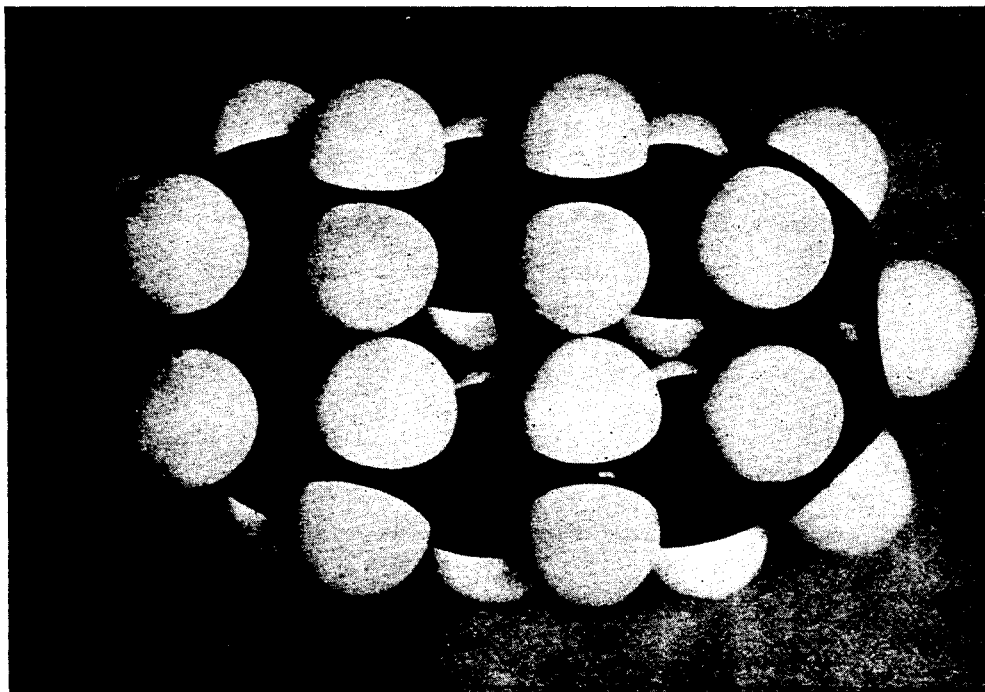
Fig. I. Cyclohexane.

Fig. 2. Cyclodecane.

Fig. 3. Cyclopentadecane.

Of the other physical properties of multimembered rings, we shall mention only briefly the melting point and the specific gravity. For purposes of comparison, Fig. 4 shows the melting point curves of the homologous series of the normal paraffin hydrocarbons and of the cycloparaffins (polymethylenes). Whereas the melting point curve of the normal paraffins exhibits the familiar regular zig-zag shape, that of the cyclic hydrocarbons is extremely irregular up to the 15-membered ring. Beginning with the 16-membered ring, the melting point curve of the cyclic paraffins also becomes regular and approximately parallel to that of the normal paraffis. This "normalisation" thus takes place in the cyclic series at approximately the same number of carbon atoms in the molecule as that at which the initially zig-zag curve of the aliphatic series assumes an approximately linear course.

The orthobaric density curves* of the normal paraffins and the cyclic hydrocarbons also exhibit characteristic differences. It can be seen from Fig. 5 that the orthobaric density curves of the individual paraffins are similar in shape,

*Experiments published in the ETH thesis of H. Wild, Zurich, 1945.

and can be made to coincide by a suitable displacement. This is not the case
with the cyclic hydrocarbons (Fig. 6). The orthobaric density curves of the
higher members are so steep on the liquid side that the individual curves inter-
sect*, and this is the reason that the densities (at a certain arbitrarily chosen
temperature) of the homologous series of cyclic compounds reach a maxi-

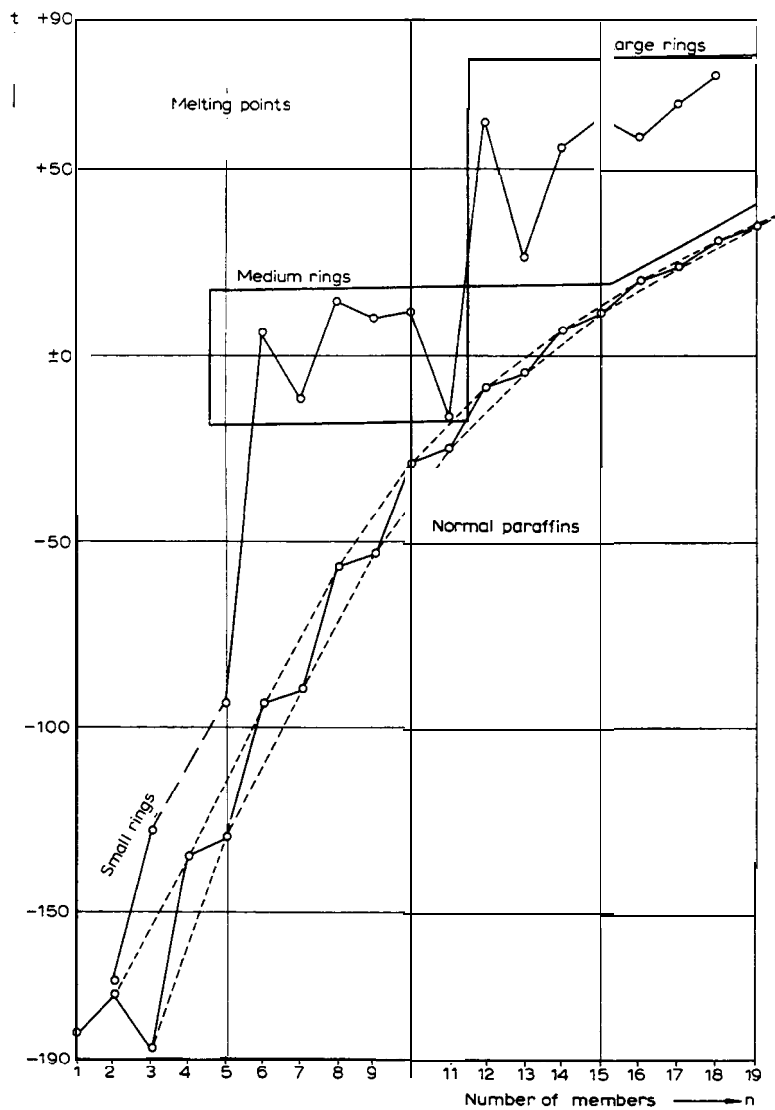* The points of intersecticn of the curves are not shown in Fig. 6.

Fig. 4. Melting-point curves of normal paraffins and cycloparaffins (polymethylenes).
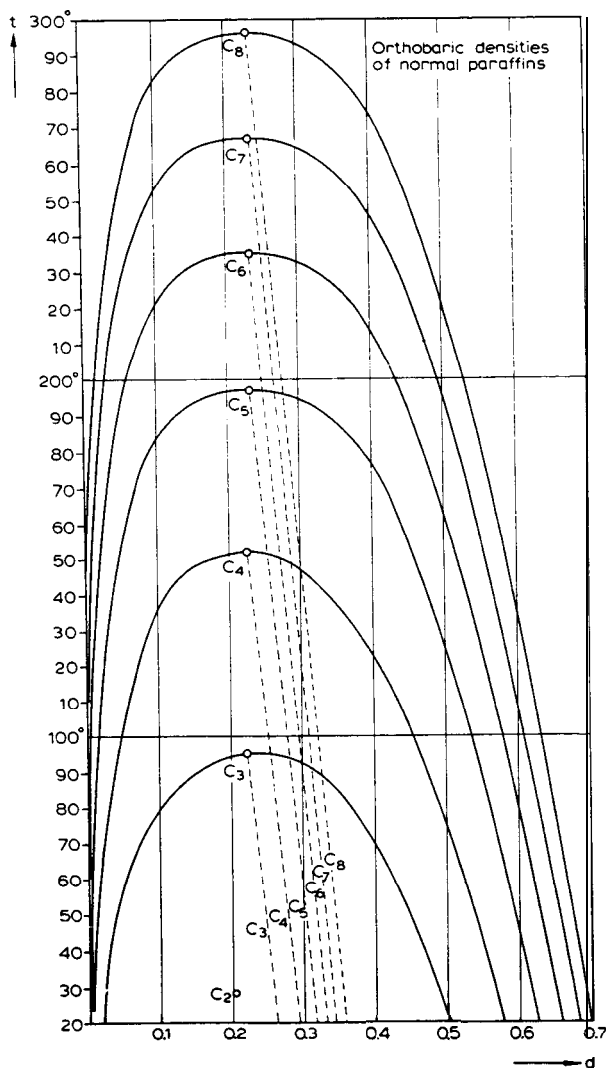
Fig. 5. Orthobaric densities of normal paraffins.

mum with a certain ring system which depends on the temperature selected. In the cycloparaffin series, for example, the maximum density at -50° is shown by cyclodecane, and that at 20° by cyclotetradecane. Only at about 150° does this maximum completely disappear, and the density curves for a given temperature above this value follow a similar course (i.e. they rise continuously with increasing molecular size) to that observed at lower temperatures for the aliphatic compounds.
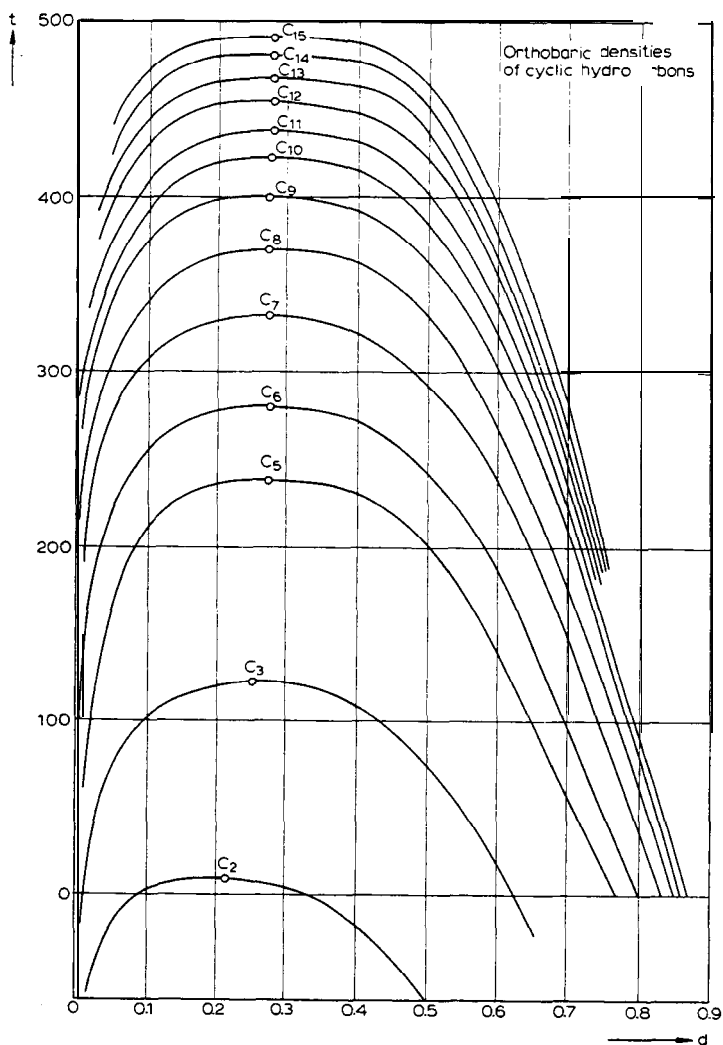
Fig. 6. Orthobaric densities of cycloparaffin.

The homologous series of cyclic ketones provides a typical example of the dependence of physiological properties on details of the molecular structure. Whereas the familiar mixed odour, between bitter almond, caraway and peppermint, occurs with the 5- to 8-membered ring ketones, the 10- to 12-membered compounds have a characteristic camphor odour. The musk odour is found with the 14- to 18-membered ketones, the optimum, in the case of the unbranched and saturated cyclic ketones, occurring with cyclopentade-

none. Numerous multimembered heterocyclic compounds prepared subsequently, such as lactones[13], esters and imines, also show a musklike odour. The optima in this series occur roughly with the compound which contains 15 carbon atoms in the ring in addition to the hetero-atom.

The first multimembered heterocyclic compounds were prepared from cyclic ketones by conventional methods of ring expansion. Thus, multimembered ketones gave the corresponding lactones (XII) when treated with Caro's acid, whilst the cyclic imines (XIII) could be prepared from the ketoximes by a Beckmann rearrangement, followed by reduction of the isoxime carbonyl to a methylene group.
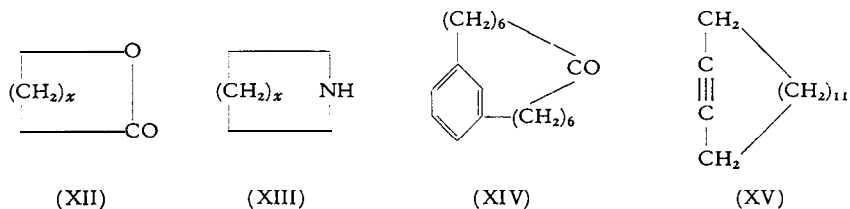
It is a well-known fact that a small ring can be fused to the benzene ring only in the *ortho*-position. It was to be expected, however, that sufficiently large rings could also be closed on the *meta*- and *para*-positions. This was in fact confirmed. As an example, we mention the first experimentally realized case, namely 6,6'- (*m*-phenylene)-dihexyl ketone (XIV).

Experience has also shown that a normal triple bond cannot exist in a small ring, since the triply bonded carbon atoms and the adjacent carbons lie in a straight line. An examination of the model shows that a triple bond should be possible in a sufficiently large ring. This assumption was experimentally confirmed by the preparation of cyclopentadecyne (XV).

Ziegler[14] later achieved a considerable improvement in the yields of multimembered cyclic ketones, by appropriate use of the principle (first reported by Paul Ruggli) of ring formation in a very dilute solution. Whereas this method permits of at least a ten-fold increase in the yields of the 15- to 17-membered cyclic ketones, the yield of cyclodecanone still remains at a minimum of a few tenths of a per cent. Multimembered heterocyclic rings's have also been prepared by means of the principle of "infinite dilution". We may recall here the first experimental achievement of the practically quantitative lactonisation[16] of 15-hydroxy-1-pentadecanoic acid. The resulting lactone

(XII, x= 14) was the same as that which had already been prepared by the action of Caro's acid on cyclopentadecanone, and which finds practical application as one of the best musk perfumes ("Exaltolid"). Cyclic imines of the type XIII have also been prepared in very dilute solution by cyclization of ω-bromoalkylamines [17].

Higher terpene compounds

A second field on which I have to report is that of the higher terpenes. My remarks are, in some respects, an extension in higher molecular spheres of the lecture delivered here in 1910 by Otto Wallach on *monoterpenes*. The structural similarity of almost all monoterpenes was at that time best expressed by regarding *p*-cymene as the aromatic parent hydrocarbon of this class. To illustrate the close relationships we show here the formula of *p*-cymene together with those of an aliphatic, a monocyclic and a bicyclic monoterpene.

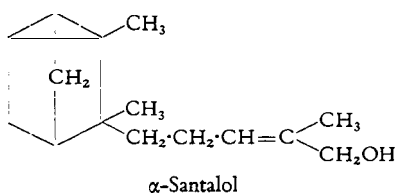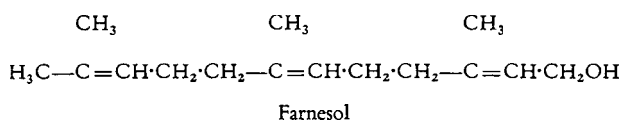*p*-Cymene   Nerol   Limonene   Camphor
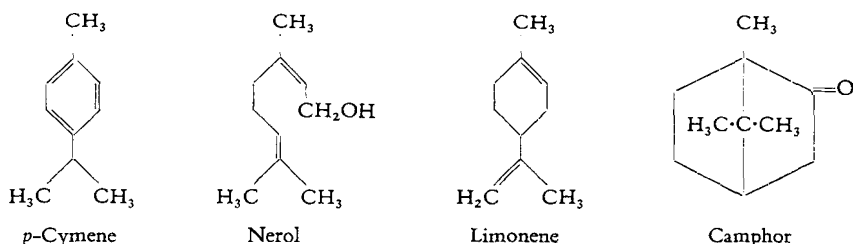
Even 35 years ago there were known compounds such as camphene and fenchone which, according to their properties, are monoterpenes, but which cannot be derived from *p*-cymene. Other monoterpenes of similar type were subsequently discovered in nature, such as artemisia ketone.

Camphene   Fenchone   Artemisia ketone
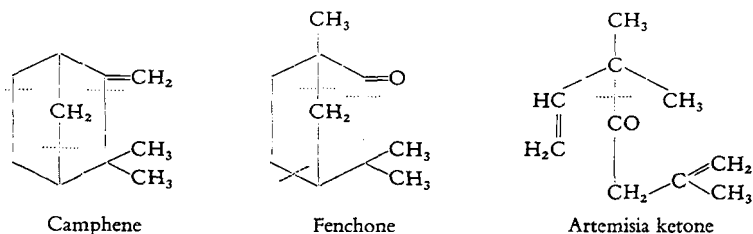
However, the carbon skeletons of this group of monoterpenes have one characteristic in common with that of *p*-cymene : they are built up of two isoprene

units*. The following is therefore proposed as a definition embracing all monoterpenes: Monoterpenes are compounds whose carbon skeleton is built up of two isoprene units.

In essential oils, the monoterpenes are frequently accompanied by compounds containing 15 carbon atoms in the molecule and closely resembling the monoterpenes in their properties. The term *sesquiterpenes* was adopted for these compounds. Prior to 1920 the constitutions of two sesquiterpene compounds had been elucidated: the aliphatic farnesol[18] and the bicyclic α-santalol[19].

$$H_3C—C=CH·CH_2·CH_2—C=CH·CH_2·CH_2—C=CH·CH_2OH$$

with CH₃ groups above each double-bonded carbon

Farnesol

α-Santalol

Diterpenes had no place in the system of organic chemistry in 1920, nor did the natural compounds with 30 carbon atoms, whose properties no longer resembled those of the mono- and sesquiterpenes but which were nevertheless occasionally referred to as *triterpenes*.
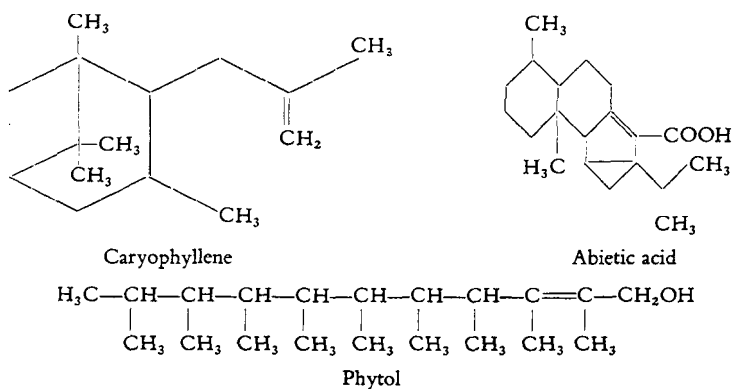
In 1920 we started work on a broad basis on sesquiterpenes, diterpenes and subsequently also on triterpenes, i.e. on compounds which may be grouped under the term "higher terpenes" or "polyterpenes". The consistent application of a simple working hypothesis and a suitable method granted an insight into the structure of the carbon skeleton, which rapidly led to a systematization of the higher terpene compounds. The working hypothesis of that time, which is nowadays known as the *isoprene rule*, was based on the tentative assumption that the carbon skeleton of the higher terpenes is built up of isoprene units.

In view of the very large number of naturally occurring higher terpene compounds, and considering their very complex structures, it was not to be expected that the usual methods of systematic degradation would lead within a reasonable interval of time to a far-reaching conclusion regarding the validity

* Indicated in the formulae by dotted lines (...).

of the isoprene rule. On the other hand, it seemed to us that dehydrogenation would probably provide a more rapid method of achieving this end. This could only be successful if a large number of higher terpenes did in fact contain hydroaromatic ring systems and could be converted, on dehydrogenation, into the polycyclic aromatic parent hydrocarbons - homologues of naphthalene, phenanthrene, chrysene, picene - since it was to be expected that the constitutions of the latter could be more rapidly elucidated, and that they could be more readily synthesized than the natural hydroaromatic compounds. It was presumed that different natural products containing the same carbon skeleton, but differing from one another in other details (for instance, double bonds and functional groups) would yield the same aromatic parent substance. We also hoped that many higher terpenes which, instead of hydrogenated benzene rings, contain other alicyclic ring systems, which in turn may have aliphatic side-chains, so that relationships similar to that of nerol, limonene, camphor or α-santalol might be expected.

We may summarize the results of our experimental work by stating that the chosen way of investigation proved very fruitful. Moreover, a glance at some of the formulae proposed prior to 1921 for higher terpene compounds, (subsequently shown to be incorrect) also illustrates the usefulness of the chosen hypothesis as a guide for a rapid advance in this large and formerly confused field. These are the formulae of the sesquiterpene caryophyllene[20] and of the diterpenes phytol[21] and abietic acid[22], which do not follow the isoprene rule:

Caryophyllene   Abietic acid

$$H_3C—CH—CH—CH—CH—CH—CH—CH—C{=}C—CH_2OH$$
$$\quad\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3\;\; CH_3$$

Phytol

We shall now review some typical examples from the chemistry of higher terpene compounds. A discussion restricted mainly to the carbon skeletons will be sufficient to indicate the general traits of the mutual relationships of these compounds.

A number of bicyclic *sesquiterpenes* gave a naphthalene hydrocarbon $C_{15}H_{18}$ on dehydrogenationas, whereas others gave the parent hydrocarbon $C_{14}H_{16}$. These observations were explained by the additional assumption that the carbon skeletons of these two subgroups of bicyclic sesquiterpenes are derived from farnesol by two formally different modes of cyclization.[24] It was actually the loss of one carbon atom in the formation of the dehydrogenation product $C_{14}H_{16}$ from the starting material containing 15 carbon atoms, which encouraged us to the hypothesis which is formulated below:

Cadalene ($C_{15}H_{18}$)

Farnesol   (with 2 double bonds)   Eudalene ($C_{14}H_{16}$)
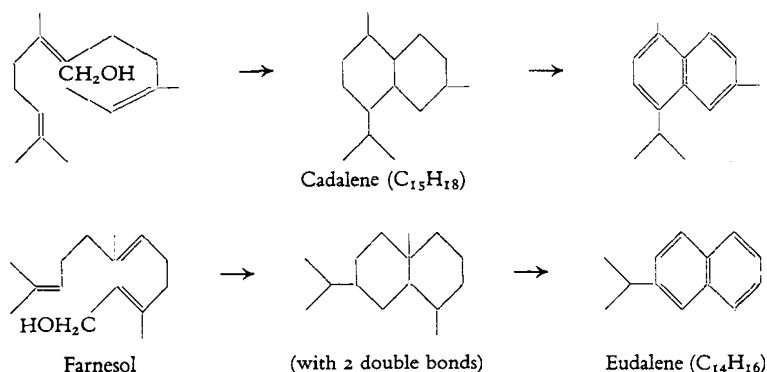
The structural formulae deduced for the two dehydrogenation products, cadalene $(C_{15}H_{18})$ and eudalene $(C_{14}H_{16})$ could be confirmed by synthesis of these hydrocarbons.

The cyclisation of farnesol into a hydrogenated naphthalene derivative of the cadalene type could also be effected in the laboratory; furthermore, we were able to isolate the hypothetical monocyclic intermediate in this transformation and to identify it as the naturally occurring hydrocarbon bisabolene $C_{15}H_{24}$


Farnesol   Bisabolene
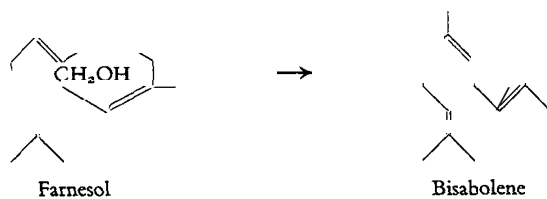
The positions. of the double bonds and of the oxygen atoms in the sesquiterpene carbon skeletons elucidated in this manner, as well as the position of the methyl-group split off during the dehydrogenation, have been determined

by degradation methods, although in some cases only with considerable effort. The first sesquiterpene compound of the eudalene type in which the postulated position of the methyl-group split off during dehydrogenation could irrevocably be confirmed is santonin[25].
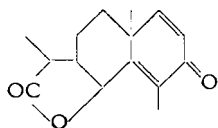

Santonin

Whereas the sesquiterpenes of the cadalene and eudalene type can be derived schematically from a regular isoprene chain, other sesquiterpenes have been discovered, which cannot be dealt with here, the formation of which could be explained by assuming an irregular condensation of isoprene units. These sesquiterpenes must be placed in an analogous class to the above-mentioned monoterpenes of irregular structure (e.g. fenchone).
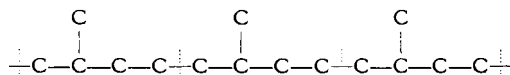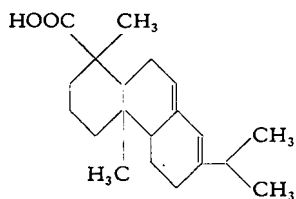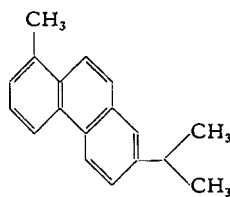

Regular isoprene chain
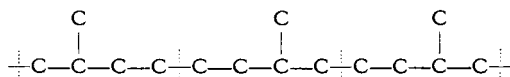
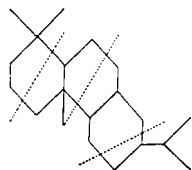Example of an irregular isoprene chain

The first compound in the series of tricyclic *diterpenes* whose carbon skeleton was elucidated has by dehydrogenation and degradation an irregular structure consisting of four isoprene units[26]. This compound is abietic acid, which occurs in rosin, and by the dehydrogenation of which Vesterberg some time ago obtained the phenanthrene hydrocarbon retene.


Abietic acid

Retene

Irregular structure of the abietic acid skeleton, built up of 4 isoprene units

In contrast to abietic acid, the majority of diterpenes can be derived from a regular chain of 4 isoprene units. We mention here examples of an aliphatic, a monocyclic, and a bicyclic natural diterpene. The tricyclic compound iso-agathic acid is an artefact which can be prepared by ring closure from agathic acid, a resin acid occurring in some copals[27].
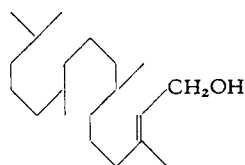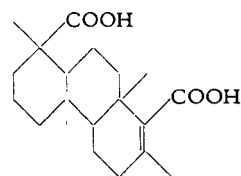

Phytol
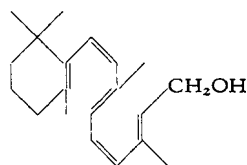
Vitamin A
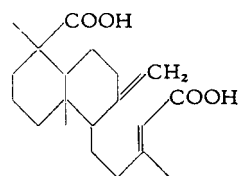
Agathic acid

Isoagathic acid

The formulae of two important natural products are shown above; these are phytol[28], which is the alcoholic constituent in chlorophyll, and vitamin A[29]. The elucidation of the constitution of these compounds, which are obtainable only in small quantities, would have met with exceptional difficulties without the guidance provided by the isoprene rule. In both cases the elucidation of the structure was facilitated by confirmation of the formula, deduced from the isoprene rule, by synthesis of either the natural product itself (in the case of phytol) or its perhydro derivative (in the case of vitamin A).

To demonstrate the complicated architecture in the *triterpene* field we will

restrict our discussion to the carbon skeleton* of the pentacyclic sub-group
of the β-amyrin type, which is widely distributed in nature.

β-Amyrin skeleton

1,8-Dimethyl-2-hydroxypicene

Sapotalene
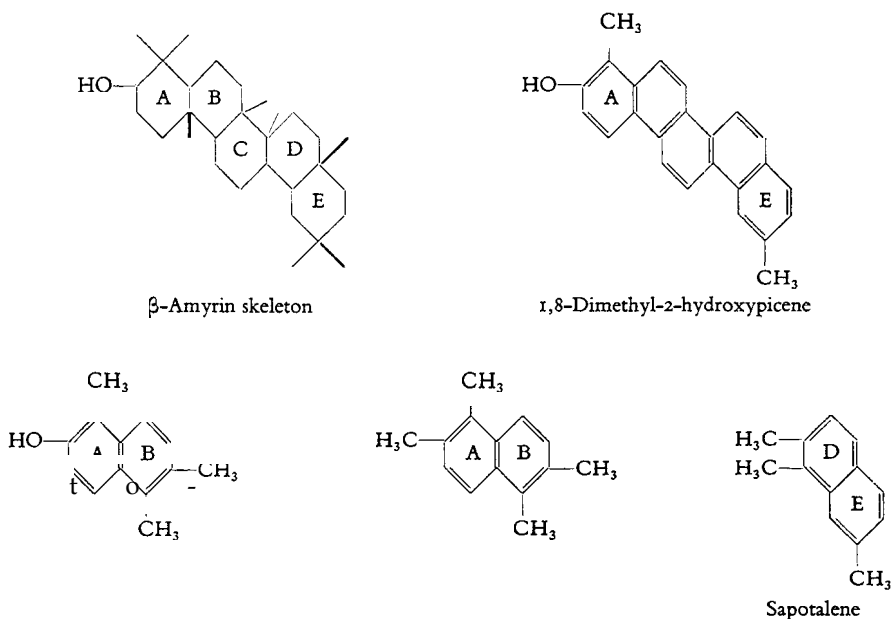
On the basis of the by synthesis elucidated dehydrogenation products, of
which the four most important are shown here, there remain only three
carbon atoms about which no information has been gained through dehy-
drogenation. These three carbon atoms are indicated by heavy lines in the
above β-amyrin carbon skeleton. However, the degradation products of
various triterpenes have directly or indirectly made it possible to localize
these three side chains, at the positions shown. The position of the double
bond was determined in a similar way. Degradation results have also yielded
further information on practically all the ring carbon atoms.

The elucidation of the carbon skeleton of the other triterpene sub-groups,
also depends on obtaining information about each individual carbon atom
on the basis of reliable dehydrogenation and degradation results.

Although the *isoprene rule* has proved an extremely valuable guide in many
cases - including the elucidation of the constitution of the carotenoids (i.e.
tetraterpenes) by Karrer and Kuhn - it nevertheless can not be regarded as an

* Containing the hydroxyl group present at the same position in almost all natural
triterpenes.

infallible law. This working hypothesis makes it possible to give precedence in experimental work to one or a few of the various carbon skeletons coming into consideration, and so to save a great deal of unnecessary work. Even if it is occasionally found, that the isoprene rule is not strictly valid for a given natural compound of a terpene type, this does not detract from its value as a working hypothesis. Experience has shown that there is no rule governing the architecture of natural compounds which is valid without exception, and which would enable us to dispense with the need to test its validity accurately for every new compound to be examined. This necessity remains, irrespective of whether the rule in question had in the past appeared to be applicable without exception, or whether exceptions had already been encountered.

Attempts may be made to interpret the isoprene rule, not only as a working hypothesis in the laboratory, but also as a structural principle employed by nature. The structural similarities of the higher terpenes raise the question as to whether these compounds may have been formed according to a uniform principle in nature[30]. At the present time, however, there is no point of reference which might lead to an interpretation of the mechanism of this biochemical process, which is so widely distributed in nature.

Male sex hormones

In addition to the terpene compounds proper, which are characterized by the presence of a number of carbon atoms divisible by 5, there exist also chemically related groups of compounds, including e.g. santene with 9 carbon atoms, the ionones with 13, and irone with 14; these may be referred to as terpenoids. Their carbon skeleton can be formally completed by addition of the missing 1-2 carbon atoms to give a basic structure that obeys the isoprene rule.
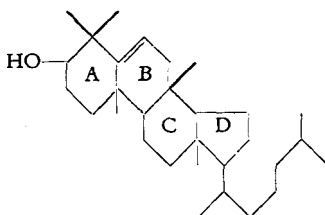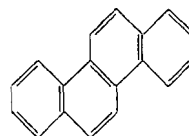

The present-day formula for cholesterol, supplemented by 3 methyl groups (--) to represent a hypothetical triterpene.

According to this view, cholesterol could be regarded as a triterpenoid[31]. On the basis of the incorrect formula which, during the years 1930-1932 - at a time when we were engaged in interpreting the results of dehydrogenation of the triterpenes - was still generally accepted as representing the structure of cholesterol*, the formation of chrysene, obtained by Diels as a dehydrogenation product of steroids, could be explained only by assuming complex and unintelligible   rearrangements.

H₃C

(+ 2 C atoms)

OH
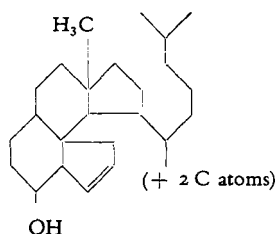
Cholesterol up to the beginning of 1932*   Chrysene

Consequently, dehydrogenation was temporarily discredited as a method of determining the carbon skeleton. Our results from the dehydrogenation of the triterpenes could be used for the determination of the carbon skeleton only on the assumption, unproved at that time, that the dehydrogenation of the steroids could be explained in a normal manner. The new formula for cholesterol, proposed and conclusively established in 1932-1933 by Bernal, Rosenheim, Wieland and Windaus, brought the desired change in the position. The formation of chrysene became readily understandable on the basis of enlargement of the 5-membered to the 6-membered ring (making use of the methyl-group).

C   D

A   B

HO
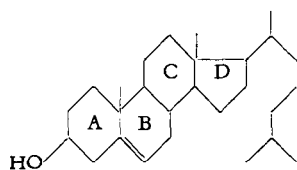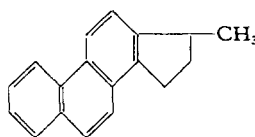
Cholesterol **   γ-Methylcyclopentenophenanthrene

This interpretation was confirmed by the fact that it explains the formation of γ-methylcyclopentenophenanthrene instead of chrysene when the dehy-

* This formula was advocated by Windaus and Wieland in their Nobel Lectures in 1928.
** Written in the manner which is presently used.

drogenation is carried out at moderately high temperatures. The migration of the methyl group is a result of the prior splitting off of the long side-chain.

At that time - from 1929 onwards - various chemists, particularly A. Bute-nandt, isolated the first sex hormones. The functional groups and, in the case of the female hormone oestrone, the structural formula - apart from stereo-chemistry - was rapidly established. In view of their tetracyclic structure, it seemed possible that the sex hormones of the oestrane and androstane type could be steroids in which the long sidechain of cholesterol had been split off. It was even conceivable the sex hormones might in the organism be formed from cholesterol by oxidative degradation of the long side chain, formally similar to the cleavage that occurs in dehydrogenation. Whether or not this assumption corresponded to reality, it was interesting to attempt the partial synthesis of a sex hormone in the laboratory by such a route. The most suitable example for this purpose was androsterone, isolated from male urine by Bute-nandt. The empirical formula of androsterone had not been accurately deter-mined by analytical methods at that time (1932/1933) but if the above as-sumption regarding the relationship of the sex hormones to cholesterol were correct androsterone would have to be assigned the empirical formula $C_{19}H_{30}O_2$. There were four known stereoisomeric saturated sterols that could be used as starting materials for a partial synthesis of androsterone. In the general formula given here for these four sterols, the 2 carbon atoms in-volved in the stereoisomerism are indicated by asterisks. By oxidation of the acetates of the four stereoisomers, we were in fact able, during the period 1932-1934, to prepare the acetates of the expected four stereoisomeric hy-droxy ketones $C_{19}H_{30}O_2$; the one obtained from epidihydrocholesterol was found to be identical with androsterone[32].

4 Stereoisomeric sterols　　　　　　　　Androsterone
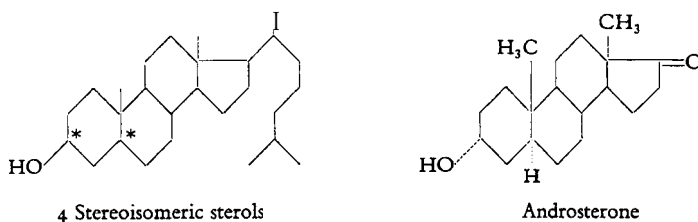
This conversion was not only the first synthesis of a sex hormone, but also the first complete structural elucidation of such a compound, and the first exact proof of the relationship between a sex hormone and a sterol - a relationship that agrees in the last stereochemical details. Afterwards such a relationship was also confirmed by other workers for the corpus luteum hormone.

Comparative physiological tests showed that androsterone must differ from the true male sex hormone occurring in the testicles, which had not been isolated in a pure form prior to 1935[34]. Even before the true testicle hormone had been isolated, we were able to gain some idea of its probable structure on the basis of the following consideration[33,36].

The method used for the partial synthesis of androsterone permitted the preparation of a series of compounds that were related chemically to andro-sterone and in part also exhibited male hormone activity. This proved that a series of androstane derivatives possess male hormone activity and it was therefore permissible to assume that the testicle hormone might also be related to androsterone. An indication of the possible position and the nature of the functional groups in the molecule of the testicle hormone was furnished by the observation, made in physiological laboratories, that the male hormone activity is destroyed when the testicle extract is treated with potassium per-manganate or boiled with alkali, whereas androsterone (and the male hor-mone activity of concentrated urine) remains unchanged when subjected to such treatments. Since unsaturated ketones containing a double bond imme-diately adjacent to the keto group are known from experience to be sensitive to alkali, we considered one of the following two formulae for the testicle hormone[33]:

(I)   (II)

Testosterone
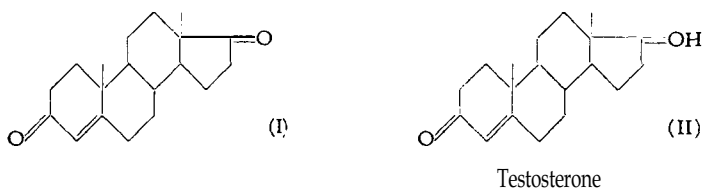
The second of these two formulae could be regarded as the more likely. It was known at the time that the reduction product of androsterone, andro-stanediol, has a greater hormone activity than androsterone itself. It was there-fore to be assumed that the second hypothetical formula for the testicle hor-mone would represent the more active compound and would therefore be that preferred by nature.

These two compounds were prepared in 1935, starting from cholester-ol[33,35,36]. At the same time, Laqueur's laboratory[34] reported the isolation of the testicle hormone from the testes of bulls. This hormone, called testoste-rone, proved to be identical with the synthetic unsaturated hydroxyketone

II, although the Dutch colleagues had at first indicated the wrong empirical formula $C_{19}H_{30}O_2$ which pointed to a stereoisomer of androsterone.

Of the more recent results obtained since 1939 in the steroid field we shall mention only one, which leads us back to a problem mentioned in the first part of this lecture.

In experiments carried out in our laboratory with the object of isolating steroids, and in particular sex hormones, of various animals, V. Prelog discovered 3α-androstenol in pig testicles. This unsaturated steroid alcohol exhibits the characteristic musk odour, which suggests a relationship between the molecular structure of this new musk perfume and that of civetone.

3α-Androstenol   Civetone
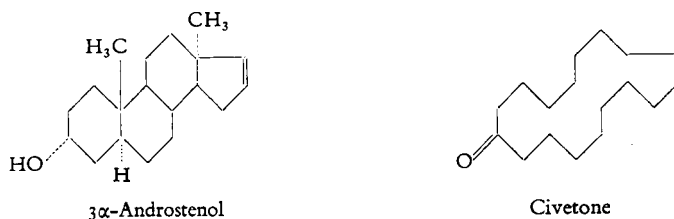
If the 3 bridges between the 4 rings in androstenol are imagined to be split, a doubly methylated 17-membered ring is obtained, in which the double bond and the oxygen atom occupy the same relative positions as in civetone.

1. W. H. Perkin, *J. Chem. Soc.,* (1929) 1347.
2. W. Markovnikov and A. Krestovnikov, *J. Russ. Chem. Phys.,* 12 (1881) 452; *Ann.,* 208 (1881) 347.
3. A. Freund, *Monatsh., 3* (1882) 625.
4. U. Sachse, Z. *Physik. Chem.,* 10 (1892) 203.
5. *Cf:* review by J. Böeseken, *Bull. Soc. Chim. France, [5]* 3 (1936) I.
6. W. Hückel and H. Friedrich, *Ann.,* 451(1926) 132.
7. L.Ruzicka, *Helv. Chim. Acta,* 9 (1926) 230 (submitted on January 26, 1924).
8. I am indebted to Ph. Chuit *[Helv. Chim. Acta,* 9 (1926) 264; cf. also later papers] for the preparation of the homologous series of the then largely unknown higher normal dicarboxylic acid.
9. Recently, Philip G. Stevens *[J. Am. Chem. Soc.,* 67 (1945) 907] detected the presence in the musk rat of 13-, 15-, 17- and 19-membered compounds, all of which could perhaps be derived from fatty acids containing from 14 to 20 carbon atoms.
10. L. Ruzicka, M. Stoll, and H. Schinz, *Helv. Chim Acta,* 9 (1926) 249. and subsequent p a p e r s .
11. L. Ruzicka and P. Schläpfer, *Helv. Chim. Acta,* 16 (1933) 162.
12. A. Müller, *Helv. Chim. Acta,* 16 (1933) 155.

13. Two multimembered lactones were detected by M. Kerschbaum, *[Ber.,* 60 (1927) 1921 in two plant oils which possess a musk-like odour, namely musk seed oil and angelica root oil. According to experience gained to date, musk perfumes of vegetable origin are lactones, whilst those of animal origin are ketones.

14. K. Ziegler and R. Aurnhammer, *Ann.,* 513 (1934) 43.

15. The preparation by W. H. Carothers [*J. Am. Chem. Soc,* 51 (1929) 2548; 55 (1933) 4714; 58 (1936) 6541 of a large number of multimembered heterocydic compounds (particularly lactones and esters) is undoubtedly also based on cyclization in a very dilute medium.

16. M. Stall and A. Rouve, *Helv. Chim. Acta,* 17 (1934) 1283.

17. L. Ruzicka, G. Salomon, and K. E. Meyer, *Helv. Chim. Acta,* 20 (1937) 19.

18. M. Kerschbaum, *Ber.,* 46 (1913) 1732.

19. F. W. Semmler, Ber., 43 (1910) 1893.

20. W. Semmler and E. W. Mayer, *Ber., 44 (1911)* 3657.

21. R. Willstätter, *Ann*., 378 (1911) 81.

22. O. Aschan and A. I. Virtanen, *Ann.,* 424 (1921) 119, 150.

23. L. Ruzicka and J. Meyer, *Helv. Chim. Acta,* 4 *(1921)* 505; 5 (1922) 345.

24. L. Ruzicka and M. Stall, *Helv. Chim. Acta, 5 (1922) 923.*

25. L. Ruzicka and A. Capato, *Helv. Chim. Acta,* 17 (1934) 614.

26. L. Ruzicka, G. B. R. de Graaf, and H. J. Muller, *Helv. Chim. Acta, 15 (1932)* 1300.

27. L. Ruzicka and J. R. Hosking, *Helv. Chim. Acta,* 14 (1931) 203.

28. F. G. Fischer, *Ann.,* 464 (1928) *69; 475 (1929)* 183.

29. P. Karrer et al., *Helv. Chim. Acta,* 14 (1931) 1431; 16 (1933) 625.

30. Added in 1965 : Such a uniform general principle is defined in the <biogenetic isoprene rule,. L. Ruzicka et al., *Experientia,* 9 (1953) 357; A. Eschemnoser et al., *Helv. Chim. Acta,* 38 (1955) 1890; L. Ruzicka, *Proc. Chem. Soc., (1959)* 341, and *Pure Appl. Chem,* (1963) 493.

31. The possibility that cholesterol might be derived from a hypothetical triterpene was one of my old cherished reveries. It was first put forward in discussion following a lecture of H. Wieland on bile acids before the Zurich Chemical Society in May 1923, and was subsequently published in the ETH thesis of my student E. A. Rudolph (Zurich, 1925). See also L. Ruzicka, *Experientia,* 9 (1953) 364. This old hypothesis has been confirmed by K. Bloch in his well-known work on the biogenesis of cholesterol.

32. L. Ruzicka, M. W. Goldberg, J. Meyer, H. Brüngger, and E. Eichenberger, *Helv. Chim. Acta,* 17 (1934) 1389.

33. L. Ruzicka and A. Wettstein, *Helv. Chim. Acta,* 18 (1935) 986.

34. K. David, E. Dingemanse, J. Freud, and E. Laqueur, Z. *Physiol. Chem., 233* (1935) 281.

35. L. Ruzicka and A. Wettstein, *Helv. Chim. Acta,* 18 (1935) 1264.

36. The first description of the procedure for the preparation of testosterone was given in a Swiss patent application from June 18, 1935 (which was the priority date for patents in many countries), and in a communication of my colleague A. Wettstein from September 10, 1935 in *Schweiz. Med. Wochschr.,* 65 (1935) 912.

    A. Butenandt and Hanisch, *Ber.,* 68 (1935) 1859, published in a preliminary note the preparation of testosterone using independently the same series of reaction steps.