CONSIDERATIONS ON THE INTERACTION OF ERGONS AND THEIR "SUBSTRATES"*

by

H. VELDSTRA

Research Laboratory, Combinatie N.V.'en Amsterdamsche, Bandoengsche en Nederlandsche Kininefabriek, Amsterdam (Netherlands)

In the field of enzymology much attention has been paid to the question of how enzymes exert their action and many an idea has been formed about the interaction between catalyst and substrate.

Generally this has led to the supposition that an intermediate enzyme/substrate complex is formed, within which the reaction proper takes place, and from which result either the final reaction products directly (MICHAELIS) or the early stages of a chain reaction (HABER-WILLSTÄTTER) (cf. the recent review by FROMAGEOT, 1946). As to the nature of the linkage between enzyme and substrate, both a truly chemical and an adsorptive fixation have been taken into consideration.

Now, the purpose of our thoughts is to ask these questions in a more general sense with respect to ergons, and especially to consider whether these "enzymatic" conceptions may prove to be applicable in a wider field, or if other mechanisms will have to be deemed possible.

Properly speaking, this problem is of a more general purport and not limited to the action of compounds designated as ergons; so a few types from other classes of physiologically active substances will also be discussed.

After it had been established that a number of vitamins (limited to the representatives of the B-group) exert a co-enzyme function, it was tempting to generalize and to direct investigations towards searching for enzymatic reactions in which the other vitamins and, perhaps, hormones could act as co-enzymes. This broader outlook is certainly rather attractive and not long ago GUGGENHEIM (1946) pointed out its advantages (cf. also GREEN, 1946). The concept of co-enzyme was extended to an organic compound of relatively low molecular weight which, combined with a specific carrier of protein character, brings about enzymatic reactions with certain substrates. This combination need not imply a more or less firm chemical bond, as in the well-known cases, but might remain merely functional.

Our investigations on plant growth regulators, especially the results obtained in a study of the relation between structure and activity (VELDSTRA, 1944), led us to consider another mode of action for these ergons. Moreover, they raised the question whether some discrimination between possible types of ergon activity is not preferable to the tendency

* English version of the text of a lecture delivered before the Section for Enzymology of the VIIth Congress of Biological Chemistry, Liège, 5-10-1946.

References p. 377

to generalize from the function of certain vitamins, which might only result in narrowing the outlook.

In enzymatically regulated reactions one is always thinking of some transformation of the substrate (oxidation/reduction, decarboxylation, transamination, hydrolytic scission and the like) and of the enzyme participating as a reactant (e.g., in transmission of hydrogen, coupling to a keto- or amino-acid, re-esterification).

Another possibility of interaction, however, is an influencing of the "substrate" (now generally to be designated as the receptor) in a more physico-chemical way, whereby it would not necessarily undergo a chemical transformation in the usual sense, but would be changed, either with regard to its energy or to certain of its physico-chemical properties—this constituting the physiological response. In such a case some action of the molecule as a whole would have to be considered more important than that of special groups taking part in a chemical reaction.

EHRENSVÄRD (1942), though in a somewhat different way, has already considered the above mentioned discrimination in a most interesting discussion of the primary events that take place on influencing a "chemoreceptor". For this „physico-chemical" type he referred to immuno-chemical investigations, such as those of ERLENMEYER and BERGER (1932), where the specific adhesion antigen/antibody is ascribed to the spatial energetical structure of both components; or, in other words, the field action of the molecule is considered to be the determining factor.

In this mode of interaction it is not the strong chemical bonds that play the most important part, but rather the weaker LONDON-VAN DER WAALS attraction forces.

PAULING (1946) is inclined to attach more importance to these weak intermolecular forces (including hydrogen-bonds) in the interpretation of many physiological processes than to the strong, intramolecular chemical bonds, which must be disrupted and united again.

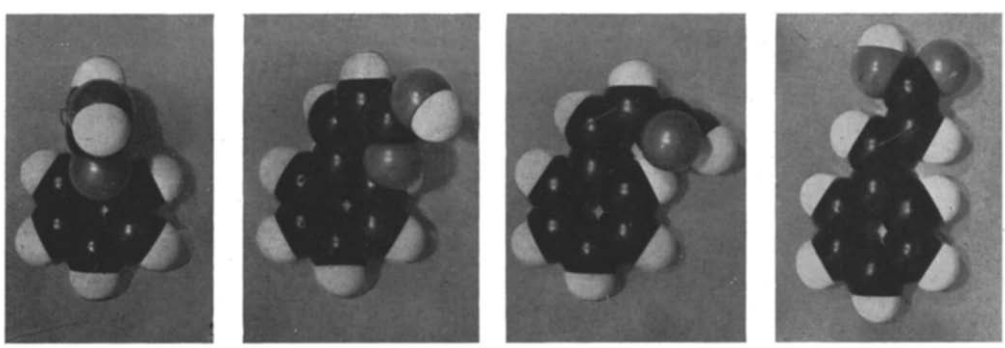


Fig. 1 *cis*-Cinnamic acid *trans*-Cinnamic acid

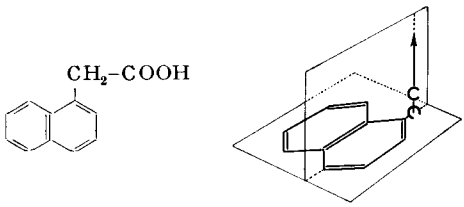
Starting from a short review of our investigations on the structure and activity of plant growth-substances, we shall proceed to explain in a wider sense our views on the action of ergons and some other types of physiologically active compounds.

The remarkable difference in activity between the stereoisomeric *cis*- and *trans*-cinnamic acids (the former shows growth-substance activity in the pea-test according to WENT (1934), but the latter does not) initiated a study of the spatial structure of these compounds with the aid of molecular models of the STUART type (1934). These models

References p. 377.

made it clear that *cis*-cinnamic acid, in contrast with the *trans*-isomer, cannot exist in a flat form (cf. Fig. 1).

This led to the supposition that the particular spatial relationship between ring system and carboxyl group required for growth-substance activity (according to KOEPFLI, THIMANN and WENT (1938)) is to be found in a COOH-group emerging as far as possible from the plane of the ring system, the "ideal" position being deemed that in which the direction of the dipole of the carboxyl group is perpendicular to this plane.



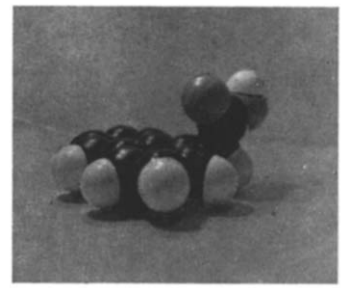
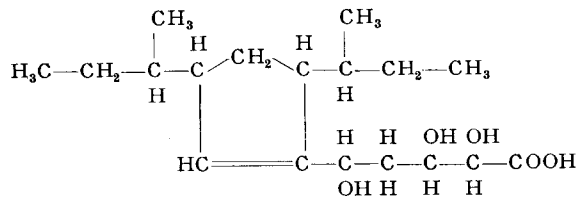


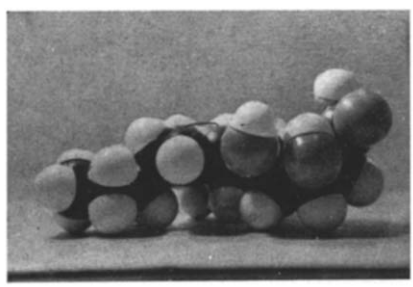
Fig. 2. α-Naphthalene acetic acid

By means of a polarographic method it was ascertained that there is a connection between the boundary activity of a number of compounds and their activity as growth-substances of such a kind that strong physiological action is always coupled with a large affinity towards the mercury/solution boundary, as measured by the suppression of the so-called oxygen-maximum (RAYMAN, 1931).

On the strength of this, the function of the nuclear ring system was considered to be that of an „attaching system", by means of which the carboxyl group—more functional with regard to the mode of action—is "served".

The combination of strong attaching power (at a boundary) with a tendency of the carboxyl group towards a peripheral position should then lead to properly active compounds. The model of the strongly active α-naphthalene acetic acid may serve as an illustration of this condensed summary of our view (cf. Fig. 2).





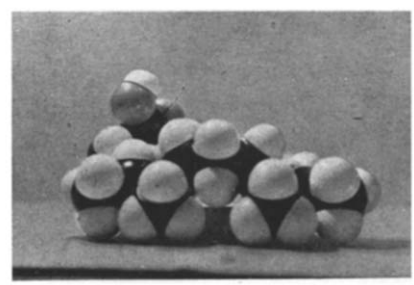


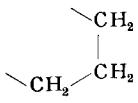
Fig. 3. Auxin a

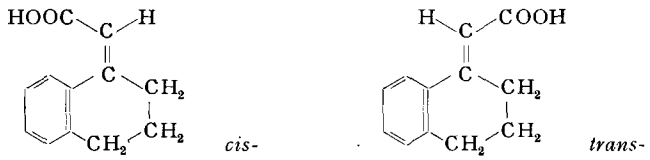
References p. 377.

It should be emphasized that this model does not represent a static configuration but only points to the possibility of an ideal position, the average statistical occurrence of which in strongly active compounds will be more frequent than in weakly active ones.

A situation such as this, with segregated lipophilic and hydrophilic parts—to be considered below in a wider sense—is shown by models, to be possible also with the natural auxins (cf. Fig. 3).

The value of this conception can be tested by the activity of numerous compounds; particularly illustrative, however, is the case of the isomeric tetralydene acetic acids. As with cinnamic acid, *cis*- and *trans*-structures are possible, which may be thought of as *cis*- and *trans*-cinnamic acids whose side-chains are fixed by the group

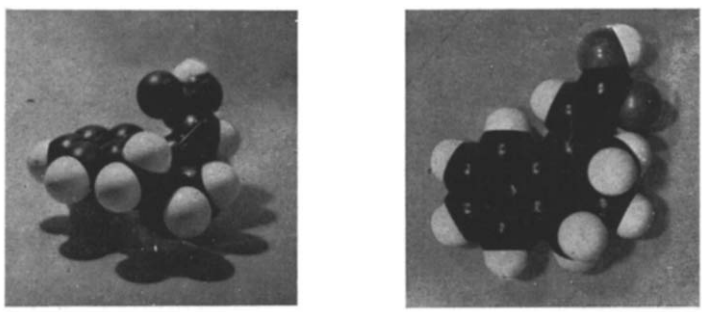




cis- *trans*-

tetralydene acetic acid

This results in a fixation of the position of the COOH-group, the *cis*-form in our opinion occupying the "active" position and the trans-isomer the unfavourable one, viz., in the plane of the nucleus (Figure 4).



cis- *trans*-

Fig. 4. Tetralydene acetic acid

Of the two forms known, melting at 92° and 163° respectively, we found the one of lower melting point to be strongly active in the pea-test, while the other substance, even at higher concentrations, showed no activity whatever. In our opinion the parallel with the case of the cinnamic acids is complete; to the substance of m.p. 92° *cis*-configuration should be attributed and to that of m.p. 163°, *trans*-configuration. Since polarographic measurements showed similar boundary activity, the difference in growth-substance activity is to be attributed to different positions of the carboxyl group.

In the light of these views on the functions of nucleus and carboxyl group in the known active compounds, we then investigated whether the role of the ring system might be filled by other structures of sufficient (lipophilic) boundary activity. For this purpose we chose the simplest type in this respect, viz., trichloroacetic acid:

References p. 377.

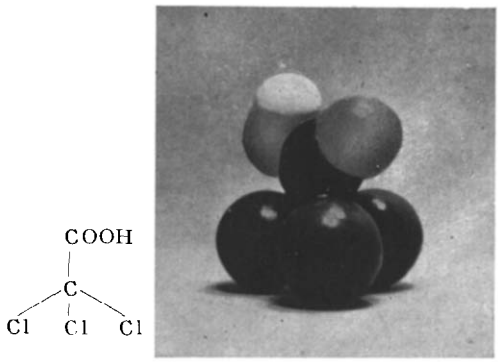
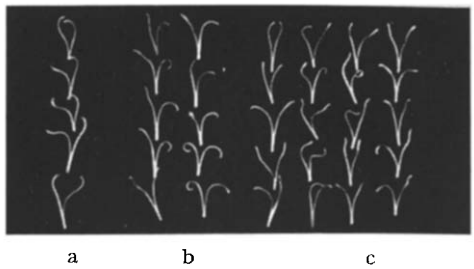


Fig. 5. Trichloroacetic acid



Pea-test trichloroacetic acid
a. α-naphthalene acetic acid 4.10^{-5} mol/l.
b. trichloroacetic acid 4.8. and $3.6.10^{-3}$ mol/l.
c. ,, ,, 9.6.; 7.2. and $4.8.10^{-3}$ mol/l.

This substance is also interesting because its carboxyl group is permanently in an "ideal" position. It proved to be indeed active in the pea-test, though to a very small extent. Apparently conditions for growth-substance activity are satisfied in principle, however, and we hold this fact in support of our conception.

The results obtained, as a matter of course, gave rise to a revision of the current opinion on the mode of action of plant growth-substances, then mostly considered as influencing the cell wall by increasing its plasticity (HEYN, 1931; SÖDING, 1931), though in recent investigations some function of the auxins in an enzymatic reaction is considered more probable (COMMONER *et al.*, 1941, 1942, 1943; BERGER and AVERY, 1943, 1944). Without going into details (cf. VELDSTRA, 1946) it looks hardly probable from a chemical point of view—considering the structure of auxins and synthetic analogues—that this function should resemble that of an oxidation-reduction catalyst in the usual sense (hydrogen carrier), as known for a number of other ergons.

The above investigations then induced us to consider whether growth-substances that are strongly lipophilic as to their "attaching" system act by becoming localized in the protoplasmic membrane (which is rich in lipophilic components, e.g., lecithin) and so

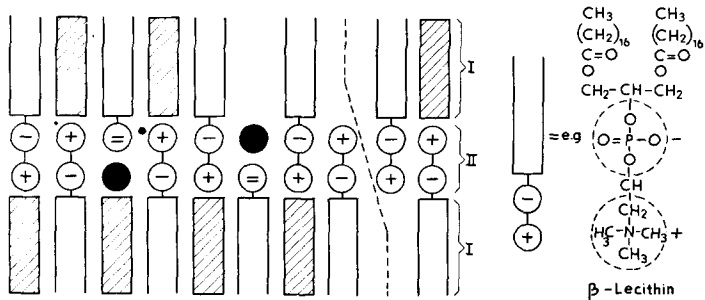


Fig. 6. Tricomplex double film of oriented phosphatide ions, as a model for the protoplasmic membrane

▨ = sensitizer molecules with condensing action (cholesterol, triolein, etc.).
From a part of the phosphatides (e.g., lecithin) choline has been split off by hydrolysis and the resulting phosphatide acid enters into mutual relation with a bivalent cation (e.g., Ca = ●).
I. Lipophilic zones: regulation of the diameter of the membrane pores by organic substances.
II. Hydrophylic zones: zone of complex relations, of selectivity and of sensitivity to p_H and electrolytes; electrical regulation of the diameter of the membrane pores.

According to BUNGENBERG DE JONG *et al.*

References p. 377.

possibly influencing its permeability, especially with respect to water and substances dissolved into it (e.g., carbohydrates), the supply of which in physiological concentrations may be increased by a turgescent action on the membrane (cf. also KONINGSBERGER, 1942). Here we relied on the fundamental investigations of BUNGENBERG DE JONG *et al.* (1942) on the structure of the protoplasmic membrane and the factors influencing its permeability (cf. Fig. 6).

The detailed argument must be omitted here (see, however, VELDSTRA, 1944) but a few items from the experiments by which this hypothesis was tested may be cited.

While KONINGSBERGER *et al.* performed experiments with isolated protoplasts, we studied (in collaboration with HAVINGA) such models as, for example, monomolecular lecithin films. In both cases interaction with growth substances was ascertained. For the present, however, these experiments are by no means complete, and definite conclusions cannot yet be drawn (cf. KONINGSBERGER, VAN DER LEK and VELDSTRA).

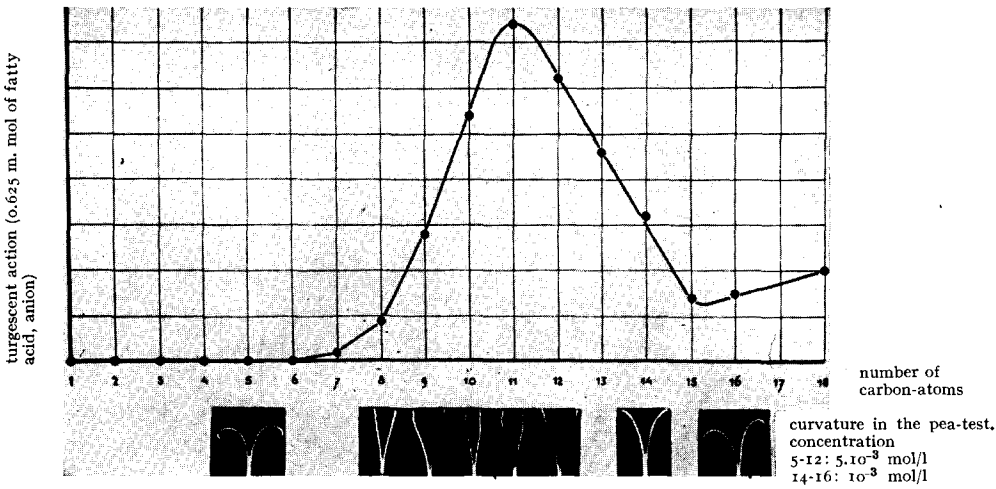


Fig. 7. Turgescent (opening) action on the oleate coacervate and growth activity in the pea-test of normal fatty acids

In collaboration with BUNGENBERG DE JONG and BOOY lipoid coacervates also were examined as model systems. In the case of the oleate coacervate a turgescent (opening) action was in fact observed with growth-substances such as naphthalene-acetic acid and indole-acetic acid. Furthermore, the inactive acetic acid proved to have a condensing action, and trichloroacetic acid, with very weak growth promoting properties, an opening effect.

Thus the expected effect proved to be present, but it also proved to be non-specific for this type of growth substances. Certain normal fatty acids, for example, showed a still stronger turgescent action with the oleate coacervate!

This led us to examine the fatty acids series in the pea-test: the acids which showed an opening action proved to be feebly active as growth-substances, and moreover there was a parallel between the intensity of the two actions!

An interesting fact is that after a certain time the action of the acids makes the objects transparent—obviously by influencing water transport—and the more so the stronger the turgescent action. These relations are recorded in Fig. 7.

References p. 377.

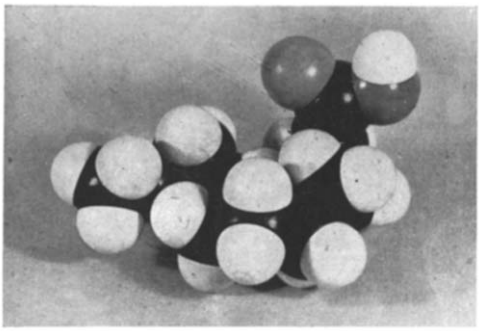


Fig. 8. Undecanoic acid, $H_3C \cdot (CH_2)_9 \cdot COOH$

The connection expected shows itself qualitatively in a very typical way, and though of course many new questions arise and extensive investigation is necessary that may not be discussed here, the observed effects look most interesting and it is ascertained that even saturated fatty acids of simple constitution, with a definite length of the chain and consequently definite physico-chemical properties, meet the essential requirements for growth-substance activity.

In our opinion this strongly suggests that growth-substance activity is of a physico-chemical nature, different from the chemical mode of action of many co-enzymes.

The train of thought just outlined makes it look probable that the "active form" of these fatty acids is not that of a stretched chain, but one "raising its head", i.e. its carboxyl group, as for instance in the model of one of the possible forms of undecanoic acid, illustrated in Fig. 8.

Analogues of these growth-substances, of a preponderantly lipophilic structure and containing a more hydrophilic group in a particular position decisive for the type of action, are to be found in various classes of physiologically active substances.

Here we think of the fat-soluble vitamin D (perhaps the fat-soluble vitamins A, E and K may have to be included to some extent), of the steroid hormones and, beyond the ergons proper, of the digitalis glucosides in some respect. Though not quite comparable, the carcinogenic hydrocarbons perhaps ought to be mentioned too.

We should like to ask the question whether a physico-chemical mode of action is to be attributed to these compounds also, but of course without confining it to influencing permeability only. Another question is whether it would be appropriate not to limit the concept of ergon action to the co-enzyme type, but rather to consider in the first instance the possibility of a different mode of action, dependent on whether the substance possesses dominantly a hydrophilic or a lipophilic structure.

In this connection it is striking that within the group of physiologically active compounds of a predominantly lipophilic structure, nearly all of them being rather inert in a chemical sense, the permitted variation in the lipophilic skeleton—while still retaining the activity of the compound—is rather large, whereas modifications of the, in general, more hydrophilic substituents immediately exert a strong influence on the activity. It looks as if the position and configuration in particular of these substituents control the intensity of the action.

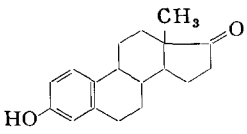
In view of these considerations, there is a possibility with a great number of synthetic compounds of reproducing qualitatively the activity of the natural lipophilic ergons, and even of equalling them quantitatively. Thus numerous substances possessing plant growth-stimulating action are known, several D-vitamins exist, a number of synthetic compounds—apparently of very different structure—show strongly oestrogenic properties, and there is also a considerable number of carcinogenic hydrocarbons.

In contrast to this, for the hydrophilic group of ergons, which are generally more

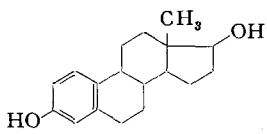
References p. 377.

chemically active, the possibilities are much more restricted; in other words, the structural specifity of the group is much higher, as for example with the water-soluble vitamins B_1, B_2, PP and C, and also in comparison with the fat-soluble vitamins E and K.

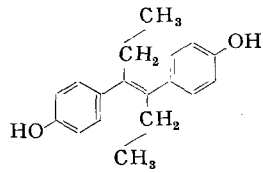
As a very instructive instance of the possibilities within the lipophilic group, we should like to point to the series of oestrogenic compounds, where the principle of the action seems to be given by hydroxy- or keto-groups placed at a well-defined distance from each other in a plane of lipophilic character (cf. SCHUELER, 1946) (threshold-values for oestrogenic activity in rats):



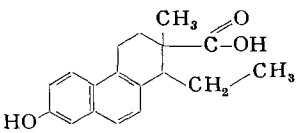
oestrone
10–15 μg



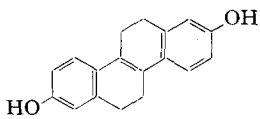
oestradiol
1–2 μg



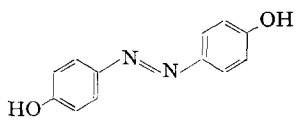
stilboestrol
2–3 μg



bisdehydro-doisynolic acid
0.1–0.15 μg

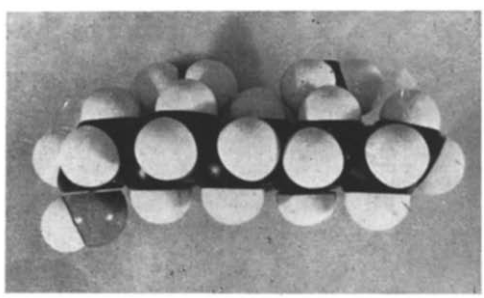


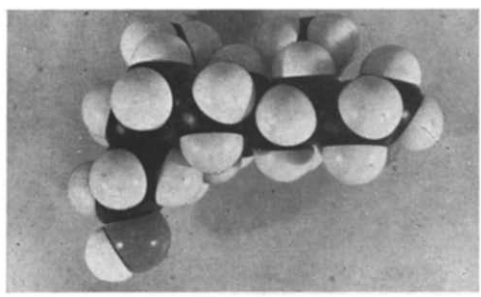
3.9-dihydroxy-5, 6, 11, 12 tetrahydrochrysene
10 μg



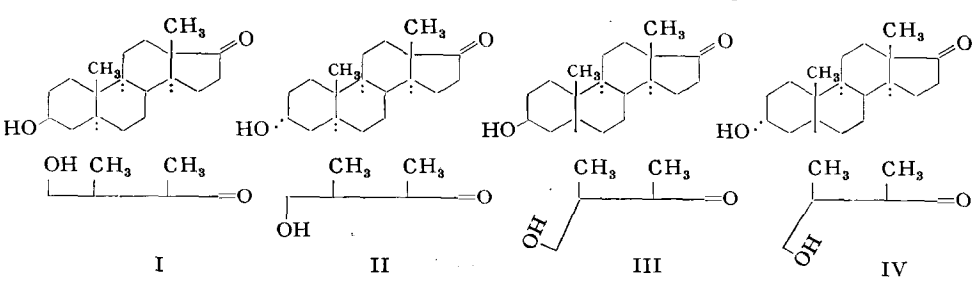
trans-p, p'-dihydroxy-azobenzene
10–15 mg

The intensity of the action is largely determined by the spatial position of these substituents. In this connection we may point to the fact, already mentioned with regard





II IV



I II III IV

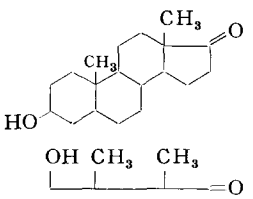
daily dose 500 μg | 70 μg | 1000 μg inactive | 1000 μg inactive

Fig. 9. Stereoisomeric forms of androstan-3-ol-17-one

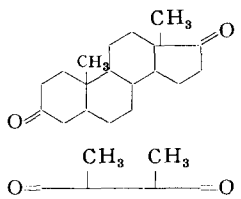
References p. 377.

to the auxins, that in the case of activity at a boundary the strongest action is induced probably by those configurations of the molecules which contain hydrophilic and lipophilic substituents on opposite side of the central plane. This flat structure, which means *trans*-configurations for all the nuclear linkages, is essential for proper activity in all the steroid hormones (cf. Fig. 9).

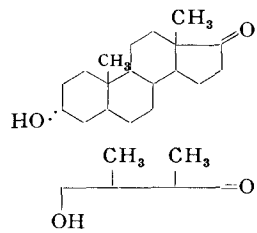
Furthermore, it is noteworthy that on comparing the positions of the lipophilic CH_3- and the hydrophilic OH-groups in a number of androstane derivatives, for instance, the activity as a male hormone is the more intense, the better the requirements of separated positions are met:



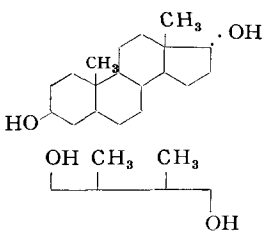
I.U. = 770 μg



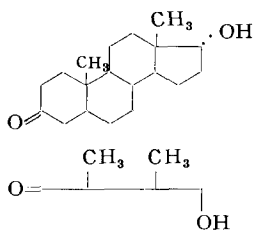
I.U. = 130 μg



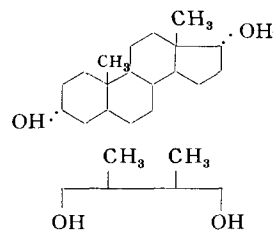
I.U. = 100 μg



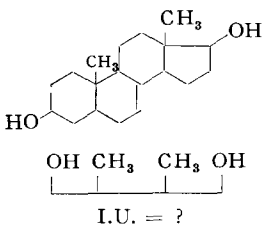
I.U. = 550 μg



I.U. = 20 μg

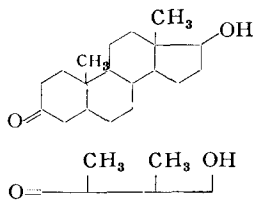


I.U. = 20 μg

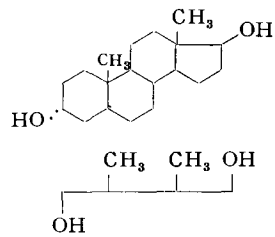


I.U. = ?

(in our opinion probably > 800 μg)



I.U. = 300 μg



I.U. = 350 μg

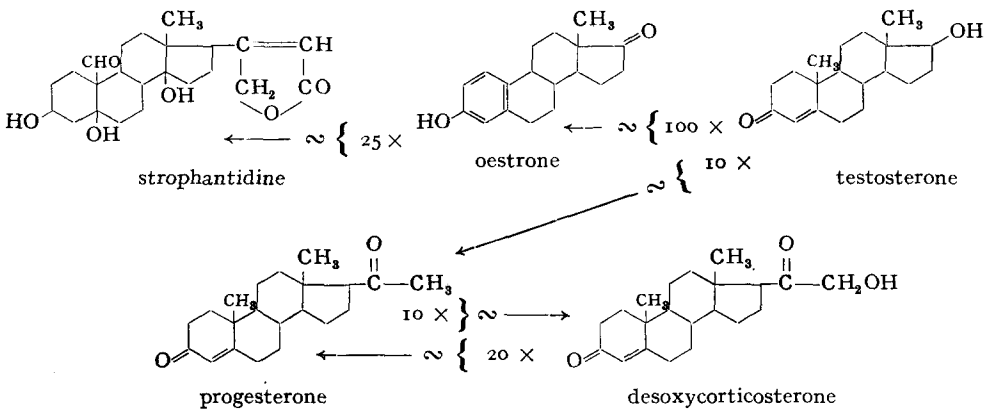
These interrelations will be analyzed in detail elsewhere; in our opinion they strongly support the view that the mode of action depends on the configuration of the molecule as a whole and not on special chemical reactions.

There are yet other phenomena which may be considered arguments for distinguishing between hydrophilic and lipophilic ergons, namely, the much larger differentiation of structure in the water-soluble compounds as compared with those in the lipophilic category, and also—probably another aspect of the same thing—the way the actions of the latter overlap one another.

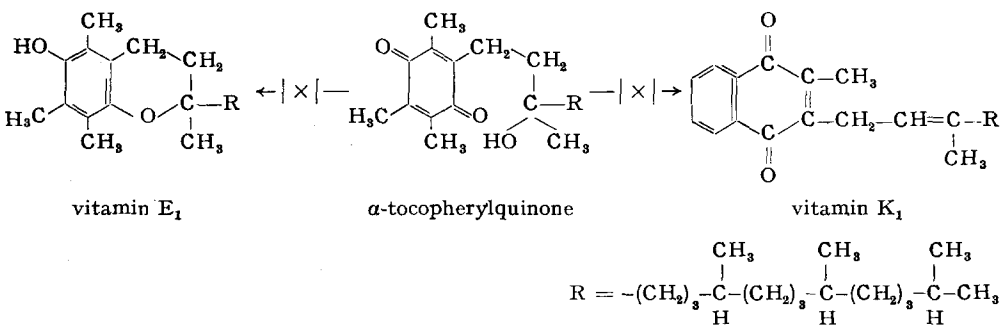
References p. 377.

Whereas it is out of the question that among the vitamins B_1, say, in very high concentration should show the action of B_2 or C, it is possible to induce growth of the uterus in a castrated animal by means of the male hormone, testosterone, provided a centuple dose is administered, as compared with the quantity of oestrone required. This type of action shows similarity to that of the other female hormone, progesterone: the latter ergon is able to keep alive adrenalectomized rats if it is given at a level ten times that of the cortical hormone, desoxycorticosterone, whereas this, in its turn, displays progesterone activity in 10- to 25-fold dosages.

Though it might be possible to suppose an *in vivo* transformation of one hormone into another, closely related chemically, there is practically no chance of this in the case of the comparative equivalence of oestrogenic hormones and cardiac glucosides, in their influence on the mechanical function of the heart in cardiac insufficiency (KROETZ, WIEBERS, 1943).



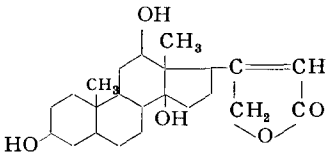
A somewhat different type of correlation of this kind between the action of diverse ergons is found in the observation by WOOLLEY (1945) that administration of α-tocopherylquinone causes deficiency symptoms which may be classed with the avitaminoses E and K.



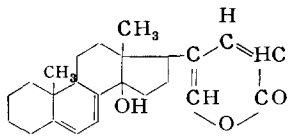
Furthermore, it is remarkable that the vitamins A and D, the fatty acids designated as vitamin F, as well as the oestrogenic hormones, contain a component in their action, that stimulates cell growth (cf. the influence on wound healing), while both digitalis glucosides and oestrone show a certain action on plant growth.

References p. 377.

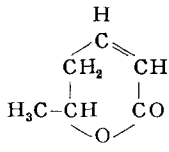
Moreover, there is a connection between the structure and activity of the cardiac glucosides (or more exactly their aglucones) and the blastocholines; the latter are known by their inhibitory action not only on seed germination but also on growth in general (comp. VELDSTRA, HAVINGA, 1943, 1944), and some of them function at the same time as the odoriferous principle of the plants in question.



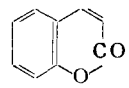
digoxigenin



scillaridin A

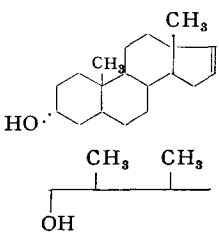


parasorbic acid (from *Sorbus aucuparia*)

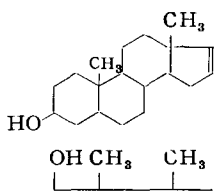


coumarin (from *Asperula odorata*)

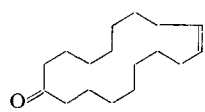
With regard to the latter fact we should finally like to mention the steroid androsten ol-3, which possesses musk odour, and the analogy between its structure and that of civetone (PRELOG, RUZICKA, 1944), as another instance of a variable skeleton with a number of characteristic groups in constant positions, and hence with corresponding action.



androsten-ol-3α



androsten-ol-3β



civetone

In the small group of steroids with odoriferous properties the steric structure is of great importance with regard to the odour, and this is influenced by the configuration in a similar way to the male hormone action (PRELOG, RUZICKA, MEISTER, WIELAND, 1945). In the case of the epimeric androstenols the musk odour of the α-compound proves to be strong, whereas that of the β-isomer is faint (cf. MIESCHER, 1946). So here too we have the strongest effect if the lipophilic and hydrophilic groups are situated on different sides of the central plane of the molecule!

In spite of all the diversity of final effects, this body of facts in our opinion very strongly suggests a feature in common with the lipophilic ergons, namely, an affinity for biological boundary structures, probably of preponderantly lipophilic character, whereby the essence of each separate action is determined by the nature of the receptor ("substrate") and the ergon "fitting" it at certain characteristic groups. (The influence of secondary factors, such as transport for example, which may play an extremely important part, is omitted here). The intensity of the adhesion of the lipophilic ergons to the receptor, determined chiefly by weak forces like LONDON-VAN DER WAALS attractions and hydrogen bonds, will depend on the "fitting", and recently HAVINGA (1946) has given a theoretical basis to this view by showing that the adhesion time is very much dependent on the number of bonds, which in turn is a question of the fitting. The energy

References p. 377.

of these attractions being estimated at ten times that of the thermal motion—which competes with the fitting— the adhesion time in a certain case on changing from two to three bonds increases from 0.001 second to 10 seconds. A fourth bond then raises the adhesion to the order of magnitude of 24 hours!

This dependence of the activity on the structure of the receptor may explain why with the lipophilic ergons true *in vitro* reactions (in a cell-free medium) are found less frequently than with the water-soluble ones, where in certain cases a well-defined chemical compound may serve as a substrate.

For this reason it will be necessary to learn more about the nature of the receptor in the cell, and this involves among other things that besides the search for enzymatic reactions, where ergons may function as co-enzymes, model systems will have to be brought into the investigations (cf. the growth substances). In this connection the results of colloidchemical researches like those by BUNGENBERG DE JONG *et al.* (1942) will in our opinion be very important. Hence the tactical approach to these problems which we have adopted throughout this lecture.

We realize clearly that many objections and unsolved questions have been treated inadequately (e.g., the feeble oestrogenic activity of *trans*-dihydroxyhexahydrochrysene; DODDS, ROBINSON, 1938). However, this should not discourage us, but on the contrary stimulate us to more detailed studies.

SUMMARY

Mainly on account of the fact that a co-enzyme function has been established for a number of vitamins, many investigators are inclined to generalize and therefore to direct their researches towards the discovery of enzymatic reactions in which the respective ergons act as co-enzymes.

Following on results obtained in a study of plant growth regulators, however, we came to consider a physico-chemical type of action to be more probable for these ergons than an enzymatic, more chemically reactive one. This action would then consist in influencing the properties of physiologically important interfaces, in this case the protoplasmic membrane.

Of the observations leading to this view the more important are discussed and demonstrated, including the growth substance activity established for certain normal fatty acids.

After extending this review to other physiologically active compounds, just as the growth substances of a predominantly lipophilic (nonpolar) structure, and mostly containing hydrophylic (polar) substituents in particular positions (as e.g., the fat-soluble vitamins, notably vitamin D, steroid-hormones, cardiac glucosides and, to a certain extent, carcinogenic hydrocarbons), the question is asked whether there is any reason why the concept of ergon action should be limited to the co-enzyme type and not broadened to include different modes of action, according to the predominance of either hydrophilic or lipophilic character in the active compound.

This question is answered on the side of broadening the concept after analysis of the above-mentioned material, which among other things points to the much smaller structural specifity of the lipophilic ergons (generally rather chemically inert) as compared with that of the hydrophilic group (of more chemically reactive compounds). Moreover, the differentiation of structure within the first group is on the whole very much smaller, and with this is probably connected overlapping of each others actions. For a certain type of action in this group (e.g., oestrogenic activity) the location of the hydrophilic substituents in the variable lipophilic skeleton, however, is extremely important.

With the steroid hormones it appears that besides a completely flat structure (trans-trans-trans linkage of the ring systems), a fact already known, the spatial position of the substituents determines the intensity of the action. This is optimal if hydrophylic and lipophilic substituents are situated on opposite sides of the central plane, resulting in a triplex layer structure of the molecule.

For the growth substances comparable requirements were deduced earlier in a different way, and now they have been established also for steroids with odoriferous properties. These facts form an additional argument for the view that these types of non-polar—polar ergons function in boundary systems.

The action of these compounds, predominantly physico-chemical in our opinion, generally seems to be more dependent on the structure of the receptor ("substrate") than that of compounds

References p. 377.

specifically soluble in water, which on the whole represent the chemical reactive type and comprise all ergons for which a co-enzyme function has been proved. The degree of interaction ergon/receptor, which finally determines the intensity of the action, appears to be extremely dependent on the number of bonds (in the lipophilic category chiefly weak forces like LONDON-VAN DER WAALS' attractions and hydrogen-bonds), which depends in turn on the "fitting" of ergon and receptor.

From these points of view the great influence of location and spatial position of the substituents then becomes intelligible.

RÉSUMÉ

Se basant sur ce qu'un rôle de coenzyme a pu être attribué à un certain nombre de vitamines, de nombreux chercheurs tendent à généraliser ce fait, et cherchent à découvrir des réactions enzymatiques dans lesquelles les ergons respectifs agiraient en coenzymes.

Des résultats obtenus au cours d'une étude sur les régulateurs de croissance des végétaux, ont montré cependant que ces ergons agissent plutôt par un mécanisme physicochimique que par un mécanisme enzymatique, essentiellement chimique. Ce mécanisme consisterait à influencer les propriétés des interfaces physiologiquement importantes, en l'occurence la membrane protoplasmique.

Les plus importantes des observations sur lesquelles s'appuie cette manière de voir sont discutées, et les conclusions qui en dérivent, démontrées en s'appuyant sur l'exemple de l'activité de facteurs de croissance présentée par certains acides gras normaux.

Après avoir étendu cette revue à d'autres composés physiologiquement actifs, comme les facteurs de croissances de caractère essentiellement lypophile (apolaires) et souvent contenant un substituant hydrophile (polaire) dans une position particulière — comme par exemple les vitamines lyposolubles, en particulier la vitamine D, les hormones stéroïdes, les glucosides cardio-toniques, et, dans une certaine mesure, les carbures d'hydrogène cancérigènes, la question se pose de savoir si le concept de l'action de l'ergon d'après le type de coenzyme est absolument général, ou s'il subsiste une possibilité d'autre mode d'action selon que domine le caractère hydrophile ou le caractère lypophile du composé actif.

L'analyse de l'action des substances citées plus haut montre que la deuxieme manière de voir doit être acceptée. Cette analyse met en évidence, entre autres choses, la spécificité structurale beaucoup plus faible lorsqu'il s'agit des ergons lypophiles (généralement assez inertes au point de vue chimique), comparée à la spécificité présentée par les groupes hydrophiles (des composés beaucoup plus actifs au point de vue chimique). En outre, la différentiation de structure dans le premier de ces groupes, considéré comme un tout, est de beaucoup la moins marquée, ce qui explique que les activités de chacun des représentants chevauchent. Considérant un certain type d'action dans ce groupe (par ex. l'activité oestrogène), la localisation des substituants hydrophiles dans le squelette lypophile est toutefois extrèmement importante.

Dans le cas des hormones stéroïdes, il apparaît que, à côté d'une structure totalement plane (union trans-trans-trans des cycles), la position spatiale des substituants est fondamentale pour déterminer l'activité. Cette dernière est maximum si les substituants hydrophiles et lypophiles sont situés sur des côtés opposés du plan central, donnant ainsi à la molécule une structure à triple couche.

De telles considérations ont pu être établies précédemment pour les substances agissant en facteurs de croissance; ces considérations peuvent être appliquées maintenant aux stéroïdes possédant des propriétés odorantes. Ces faits constituent un argument supplémentaire en faveur de la manière de voir que les types d'ergons (non polaires-polaires) agissent aux interfaces.

L'action de telles substances, s'exerçant surtout par un mécanisme physico-chimique, semble dépendre plus de la structure du récepteur que l'action des composés nettement solubles dans l'eau, lesquels dans l'ensemble appartiennent au type chimiquement actif, et comprennent tous les ergons pour lesquels a été établi un rôle de coenzyme. L'intensité de l'interaction ergon-récepteur qui finalement détermine l'intensité de l'ergon, dépend de façon étroite du nombre de liaisons qui se manifestent au cours de l'adaptation de l'ergon et du récepteur (dans le cas des substances lypophiles il s'agit principalement de force d'attraction du type LONDON-VAN DER WAALS, et de ponts d'hydrogène).

Ces différentes manières de voir permettent de comprendre l'influence considérable exercée par la localisation et la position spatiale des substituants.

ZUSAMMENFASSUNG

Hauptsächlich auf Grund der Tatsache, dass eine Co-Enzymfunktion für eine Anzahl Vitamine nachgewiesen werden konnte, sind viele Forscher zu Verallgemeinerungen geneigt und streben deshalb nach der Entdeckung von enzymatischen Reaktionen, bei welchen die betreffenden Ergone als Co-Enzyme auftreten können.

Infolge der Ergebnissen, die bei einer Untersuchung über Pflanzenwuchsregulatoren erhalten

References p. 377.

wurden, kamen wir jedoch dazu, einen physikalisch-chemischen Wirkungstyp für die betreffenden Ergone als wahrscheinlicher zu erachten als einen enzymatischen, mehr chemisch-reaktiven. Diese Wirkung würde dann in der Beeinflussung der Eigenschaften von physiologisch wichtigen Grenzflächen bestehen, in diesem Falle Protoplasmamembranen.

Von den zu dieser Anschauung führenden Beobachtungen werden die bedeutenderen diskutiert, und demonstriert, u.a. bei der Wuchsstoffaktivität, die für gewisse normale Fettsäuren festgestellt wurde.

Nach der Erweiterung dieser Übersicht auf andere physiologisch aktive Verbindungen — wie die Wuchsstoffe mit vorherrschend lipophiler (unpolarer) Stuktur, die meistens hydrophile (polare) Substituenten in einer besonderen Position enthalten, wie z.B. die fettlöslichen Vitamine, besonders Vitamin D, Steroidhormone, Herzglukoside und zu einem gewissen Grade carcinogene Kohlenwasserstoffe — wird die Frage gestellt, ob Gründe vorhanden sind, um die Auffassung der Ergonwirkung nicht auf den Co-Enzymtyp zu beschränken, sondern die Möglichkeit verschiedener Wirkungsarten zu betrachten, entsprechend dem Vorherrschen von entweder dem hydrophilen oder dem lipophilen Charakter in der aktiven Verbindung.

Diese Frage muss bejaht werden nach einer Analyse der obenerwähnten Materials, das u.a. die weitaus geringere strukturelle Spezifität der lipophilen Ergone (im allgemeinen chemisch ziemlich inert) aufzeigt im Vergleich zu der hydrophilen Gruppe (der chemisch aktiveren Verbindungen). Ausserdem ist die strukturelle Differenziierung innerhalb der ersterwähnten Gruppe als Ganzes weitaus geringer, womit wahrscheinlich die gegenseitige Wirkungsüberschneidung zusammenhängt. Für einen gewissen Wirkungstyp in dieser Gruppe (z.B. oestrogene Aktivität) ist die Position der hydrophilen Substituenten in dem veränderlichen lipophilen Skelett jedoch äusserst bedeutend.

Bei den Steroidhormonen zeigt sich, dass ausser einer vollkommen ebenen Struktur (trans-trans-trans-Bindung der Ringen), was bereits bekannt war, die räumliche Anordnung der Substituenten für die Intensität der Wirkung entscheidend ist. Diese ist optimal, wenn die hydrophilen und lipophilen Substituenten an entgegengesetzten Seiten der Zentralebene gelegen sind, sodass eine Dreischichtenstruktur des Moleküls resultiert.

Für die Wuchsstoffe waren vergleichbare Erfordernisse bereits früher auf andere Art abgeleitet worden und dies konnte jetzt auch für die Steroide mit Geruchseigenschaften festgestellt werden. Diese Tatsachen bilden ein zusätzliches Argument für die Ansicht, dass diese Typen von unpolar-polaren Ergonen in Grenzflächensystemen wirken.

Die Wirkung dieser Verbindungen, unserer Meinung nach vorherrschend physikalisch-chemisch, scheint im allgemeinen mehr von der Struktur des Rezeptors ("Substrat") abzuhängen, als die der spezifisch wasserlöslichen Verbindungen, die im grossen ganzen den chemisch reaktiven Typ darstellen und die alle Ergone enthalten, für welche eine Co-Enzymfunktion nachgewiesen ist. Der Grad der Wechselwirkung Ergon/Rezeptor, der letzten Endes für die Wirkungsintensität entscheidend ist, hängt, wie sich zeigt, in äusserst starkem Masse von der Anzahl Bindungen ab (bei der lipophilen Kategorie hauptsächlich schwache Kräfte, wie LONDON-VAN DER WAALS'sche Anziehungskräfte und Wasserstoffbrücken), die durch das "Zueinanderpassen" von Ergon und Rezeptor mitbestimmt werden.

Von diesem Standpunkt aus gesehen, wird der grosse Einfluss der Lage und räumlichen Anordnung der Substituenten verständlich.

REFERENCES

J. BERGER AND G. S. AVERY JR., *Am. J. Bot.*, 30 (1943) 297.
J. BERGER AND G. S. AVERY JR., *Science*, 98 (1943) 454.
J. BERGER AND G. S. AVERY JR., *Am. J. Bot.*, 31 (1944) 11.
H. G. BUNGENBERG DE JONG, survey in V. J. KONINGSBERGER *et al.*, *Leerboek der algemeene plantkunde*, Amsterdam 1942, p. 175, cf. K. C. WINKLER AND H. G. BUNGENBERG DE JONG, *Arch. néerl. Physiol.*, 25 (1941) 431, 467.
B. COMMONER AND K. V. THIMANN, *J. Gen. Physiol.*, 24 (1941) 279.
B. COMMONER AND D. MAZIA, *Plant. Physiol.*, 17 (1942) 682.
B. COMMONER, S. FOGEL AND W. H. MULLER, *Am. J. Bot.*, 30 (1943) 23.
E. C. DODDS, L. GOLDBERG, W. LAWSON AND R. ROBINSON, *Nature*, 141 (1938) 247.
G. EHRENSVÄRD, *Acta physiol. Scandinavica*, 3, Suppl. IX.
H. ERLENMEYER UND E. BERGER, *Bioch. Ztschr.*, 252 (1932) 22. cf. *ibid.* 255 (1932) 429 and BERGER, *Klin. Wochenschr.*, 14 (1935) 1377.
CL. FROMAGEOT, *Produits pharmaceutiques*, 1 (1946) 61.
D. E. GREEN, *Biochemistry from the standpoint of enzymes, Currents in biochemical research*, New York 1946, p. 149 ff.
M. GUGGENHEIM, *Experientia*, 2 (1946) 48.
E. HAVINGA, *Kennis en begrip in de organische chemie. Inaugural lecture, Leiden* 1946. cf. E. HAVINGA, H. VELDSTRA, *Rec. trav. chim.*, 66 (1947) 285.
A. N. J. HEYN, *Rec. trav. bot. néerl.* 28 (1931) 113

J. B. KOEPFLI, K. V. THIMANN AND F. W. WENT, *J. Biol. Chem.*, 122 (1938) 763.
V. J. KONINGSBERGER, *Natuurkundige Voordrachten Diligentia*, 20 (1942) 13.
V. J. KONINGSBERGER, H. A. A. VAN DER LEK AND H. VELDSTRA, *Monographs on the progress of Research in Holland. Investigations on phytohormones*, Amsterdam. In press.
CH. KROETZ AND W. WIEBERS, *Munch. Med. Wochenschr.*, 90 (1943) 203.
K. MIESCHER, *Experientia*, 2 (1946) 237.
L. PAULING, *Chemical and Engineering News* 24 (1946) 1788.
V. PRELOG AND L. RUZICKA, *Helv. chim.*, Acta 27 (1944) 61.
V. PRELOG, L. RUZICKA, P. MEISTER AND P. WIELAND, *Helv. chim. Acta* 28 (1945) 618.
B. RAYMAN, *Collection Czechoslovak Chem. Comm.* 3 (1931) 314.
F. W. SCHUELER, *Science*, 103 (1946) 221.
H. SÖDING, *Jahrb. f. wiss. Bot.*, 74 (1931) 127.
H. A. STUART, *Z. physik. Chemie B*, 27 (1934) 350; cf. *ibid.* 36 (1937) 155.
H. VELDSTRA, *Enzymologia*, 11 (1944) 97, 137.
H. VELDSTRA AND E. HAVINGA, *Rec. trav. chim.*, 62 (1943) 841.
H. VELDSTRA AND E. HAVINGA, *Enzymologia*, 12 (1945) 373.
H. VELDSTRA, *Landbouwkundig Tijdschrift*, 58 (1946) 483.
F. W. WENT, *Proc. Akad. Wetensch. A'dam*, 37 (1934) 547.
D. W. WOOLLEY, *J. Biol. Chem.*, 159 (1945) 59.

Received April 12th, 1947