Some further dehydrogenases could be demonstrated in the cells of different fungi: (e) Pentose metabolism[4]: aldopentose reductase in *Penicillium expansum* and *O. lactis* and pentitol dehydrogenase in *P. expansum* and *O. lactis*. (f) Other dehydrogenases: L-amino acid: O_2 oxidoreductase (E.C. 1.4.3.2) in *N. crassa*[3], D-glucose: O_2 oxidoreductase (E.C. 1.1.3.4) in *Aspergillus niger*[5], pyridoxol: NADP oxidoreductase (E.C. 1.1.1.1) in *S. cerevisiae*[6], glutathione reductase (E.C. 1.6.4.2) in *S. cerevisiae*[7], as well as NAD- and NADP-linked proline dehydrogenases, aldehyde dehydrogenases and dihydrolipoic dehydrogenase (E.C. 1.6.4.3) in *S. cerevisiae*[8].

The specificity of the different staining reactions could be confirmed by means of control reactions[1]. Table I shows the inhibitors employed and their concentrations which inhibited enzyme activity and thus formazan production in the cells of *S. cerevisiae*.

(2) *Oxidases.* Cytochrome oxidase and peroxidase can be demonstrated cytochemically in the cells by various indicators that are changed by oxidation to more or less stable, coloured reaction products[9].

(a) Cytochrome oxidase: After testing the well-known indicators, the combination of the 2 amines *p*-aminodiphenylamine and *p*-methoxy-*p'*-aminodiphenylamine proved to be the best method for the intracellular demonstration of this enzyme in *N. crassa*, *O. lactis* and *S. cerevisiae*[10]. After an incubation period of 30 min, the uncoloured cytoplasm contained brown-red, round-shaped deposits (Figure 2).

(b) Peroxidase: Different methods (amines, phenols, zinc-leuco) were tested, but only the indicator 3-amino-9-ethylcarbazole produced a useful reaction picture (incubation period for all fungi: 60 min): small, round, red-brown, distinct granula, equally distributed in a lightly tinged cytoplasm[10].

The results of control reactions (inhibitors with their active concentration in the medium) are summarized in Table II:

The reaction pictures of all cytochemical enzyme demonstrations showed an equal distribution of the different deposits in the cytoplasm. In the hyphae-producing fungus *N. crassa* the reactions were weaker in older parts of the mycelium than in the younger, growing hyphal tips. In the stained preparations of *O. lactis* and *S. cerevisiae*, many cells were only weakly stained or not at all. Probably, this is due to a different enzyme equipment of the single cells[11].

The practical value of the cytochemical demonstration of dehydrogenases and oxidases in fungi could be demonstrated when studying the influence of phenylboric acid on the cells of *O. lactis*[12].

Zusammenfassung. Die Ergebnisse der bisherigen Untersuchungen zum zytochemischen Nachweis verschiedener Dehydrogenasen und Oxydasen in den Zellen von Pilzen (*Neurospora crassa, Oospora lactis, Saccharomyces cerevisiae* u.a.) werden beschrieben.

J. REISS

Institut für spezielle Botanik der Universität, 65 Mainz (Germany), 8 March 1968.

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DISPUTANDUM

Relationship Between the Structure of Chloramphenicol and its Action upon Peptide Synthetase

The antibiotic chloramphenicol (CM) is a growth inhibitor and a widely used biochemical research tool because it inhibits specifically protein biosynthesis. Hundreds of chemical derivatives of CM have been tested for antibacterial activity and a set of empirical structure-activity rules has been derived from this body of chemical and microbiological information[1,2]. A few growth-inhibitory derivatives of CM have more recently been tested in vitro in ribosomal amino acid incorporation systems[3,4].

Evidence accumulating during the past years[5-7] and especially recent studies of GOTTESMAN[8] suggest that CM interferes with the action of ribosomal peptide synthetase(s), i.e. with the enzymatic transfer of growing peptides to the α-amino groups of incoming amino acids. The presence of at least 1 peptide or amide bond, such as that of acetyl-phenylalanyl-tRNA, is required for recognition and transfer of a peptide by the enzyme[8]; peptidyl synthetase appears to be an integral constituent of ribosomes[8,9].

This report suggests that CM, a substituted amide reminiscent of a dipeptide[10] (Figure 1), attaches vicariously to the peptidyl recognition site of peptide synthetase(s) and inhibits peptidyl transfer, viz. the elongation reaction in protein synthesis.

This hypothesis first predicts that chemical alterations which eliminate the amide bond of CM or affect its reactivity or steric environment will abolish activity. In-

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deed: (1) hydrolysis of the amide bond yields, besides dichloroacetic acid, the 'CM base' whose activity is less than 2% of that of CM itself[11]. (2) Substitutions for the amide hydrogen atom or the hydrogen atom of carbon 2 of the propanediol chain yield non-active compounds[12,13]; COLLINS et al.[14] have suggested that replacement of either hydrogen atom by methyl hinders sterically an essential juxtaposition between CM and its biological site of action. (3) Proton resonance spectroscopic studies of the conformation of CM in solution[15] have shown that the amide-bonded acyl side chain in CM is in a unique conformation relative to the pentacyclic hydrogen-bonded propanediol moiety. This conformation cannot be accommodated in the 3 non-active stereoisomers of CM (Figure 2) and may be necessary for the sterically unhindered access of the amide grouping to the peptidyl recognition site of the enzyme.

Structural requirements for activity concerning the electronegative acyl side-chain and the aryl moiety of CM are less rigorous and specific[1-4]; these parts of the antibiotic molecule probably engage in non-specific binding to enzyme molecules, giving rise to general toxicity of all 4 isomers at high concentrations.

Since peptidyl synthetase is considered a constituent of ribosomes[8], the above hypothesis secondly predicts that attachment of CM, but not of its non-active stereoisomers, to the peptidyl recognition site of the enzyme should be observed experimentally as specific binding of CM to ribosomes of sensitive organisms. Such detailed observations have been reported[16,17]. An estimate that 1 molecule of CM binds on the average to 1 ribosome[17] suggests that each ribosome contains 1 enzyme molecule with 1 peptidyl recognition site.

Thirdly, the above hypothesis predicts that CM should be more strongly inhibitory on the peptide elongation reaction in which the synthetase recognizes preexisting peptide or amide bonds than upon the in vitro condensation of dipeptides from 2 amino acyl-tRNA molecules, a process in which artificial initiation is enforced through the use of homopolymeric messengers and high magnesium ion concentrations. JULIAN[18] has, indeed, shown that in a ribosome-polyadenylic acid system, CM inhibits preferentially the elongation of lysyl peptides rather than the initial formation of lysyl-lysine from lysyl-tRNAs.

With current methods it is difficult to design experiments which test the proposed hypothesis more directly than has already been accomplished by the verification of certain predictions above which follow from this hypothesis. In particular, testing for inhibition of peptide synthetase by CM in competition with a peptidyl donor would require an experimental system even more highly resolved than the formylmethionyl-tRNA fragment system of MONRO and MARCKER[9]; in order to demonstrate peptidyl transfer, this system must contain at least 30% ethanol. If further resolution should necessitate further departure from physiological conditions, the general significance of test results with CM would be open to question.

An alternate hypothesis[19] has attempted, also, to relate the structure of CM to the antibiotic's action upon peptide synthetase by assuming antagonism by CM of the peptidyl acceptor rather than of the peptidyl donor: CM was envisioned[19] as a structural analog and functional antagonist of the amino acyl-adenosyl terminus of amino acyl-tRNAs.

Numerous substituted ring systems can replace the *p*-nitrophenyl moiety of CM, giving rise to highly active compounds[1,2,20]; this class of CM derivatives weakens collectively the impression of an analogy between *p*-nitrophenyl and heterocyclic bases of nucleotides[15] or nucleosides[19]. The alternate hypothesis predicts[21] that CM will

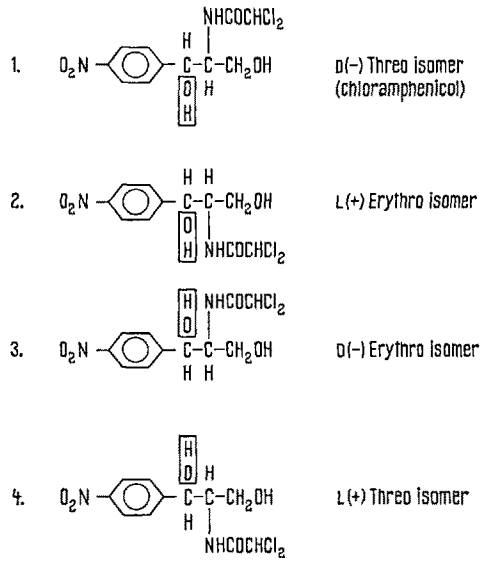

Fig. 2. Chloramphenicol and its stereoisomers.

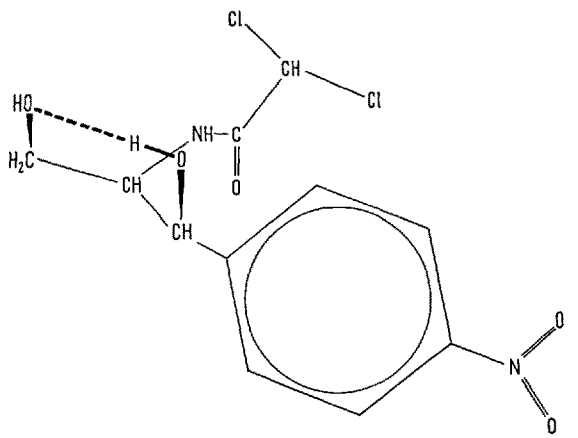

Fig. 1. Structure of chloramphenicol projected from a three-dimensional Fourier plot[26], the H-atoms were added arbitrarily since their exact positions have not been determined.

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inhibit the transfer reaction of peptides to puromycin in competition with the peptidyl acceptor, puromycin; this, however, is not the case[22]. Finally, functional antagonism between amino acyl-tRNAs and CM should logically result in inhibition of all peptide bond syntheses in which amino acyl-tRNA is a reactant rather than in the preferential inhibition of the elongation reaction that CM does produce[18,23].

Concerning the central problem, viz. the mechanism of CM's inhibition of protein biosynthesis in susceptible cells, DAS et al.[24] have reported that CM also inhibits the elongation reaction in *Escherichia coli*; the authors have also focused upon the structural relationship between CM and the C-terminal peptide grouping of nascent protein chains as a potential structural basis of CM's action. Moderately high ($1.6 \times 10^{-4} M$) concentrations of CM cause the accumulation of acid-soluble peptides in protoplasts of *Bacillus megaterium*, while CM at $1.25 \times 10^{-3} M$ completely inhibits incorporation of amino acids[25]; these observations are remindful of the differential effects of CM on initiation and elongation of peptides in vitro[18].

Zusammenfassung. Zusammenhänge zwischen Struktur und Wirkung bei Chloramphenicol führen zu der Hypothese, dass das Antibiotikum die Peptidsynthetase der Ribosome dadurch hemmt, dass es den Peptidylpartner der Reaktion antagonisiert. Im Einklang damit steht, dass Chloramphenicol sich spezifisch an Ribosome bindet und selektiv die Elongationsphase der Proteinsynthese hemmt.

F. E. HAHN

Department of Molecular Biology, Walter Reed Army Institute of Research, Washington (D.C. 20012, USA), 5 April 1968.

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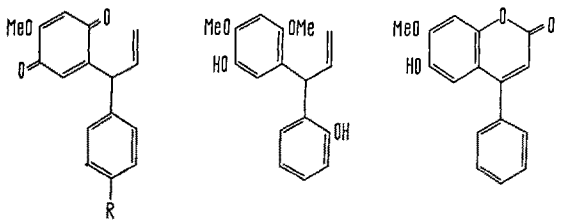
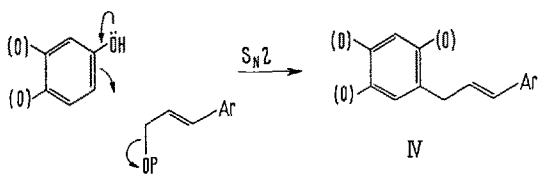
STUDIORUM PROGRESSUS

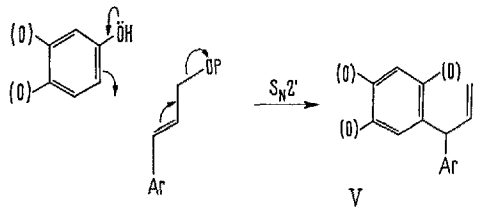
Biogenetic-Like Syntheses of Benzylstyrenes and Neoflavanoids

It is generally considered that flavanoid and isoflavanoid biosynthesis involves initial C-cinnamoylation of a phenolic C_6 unit (or its polyketide equivalent), the chalcone thus formed being cyclized, epoxidized[1] or reduced[2] to furnish the progenitors of the different types of natural flavanoids and isoflavanoids. The biosynthetic origin of neoflavanoids, viz. dalbergiones[3,4] I and related quinol derivatives, e.g. latifolin[5] II, and 4-aryl-coumarins, e.g. dalbergin[6] III, is obscure, although it has been suggested that these also might be derived from the chalcone intermediates by a double 1,2-shift of the B aryl ring[7] or a single 1,3-shift of the A aryl ring[8].

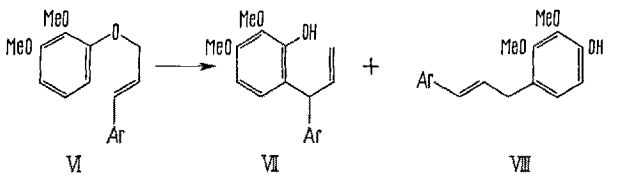


OLLIS and his associates recently reported[9] that benzylstyrenes IV co-occur with neoflavanoids V and they proposed that, in contrast to flavanoid biosynthesis, the formation of IV and V may involve S_N2 type alkylations of polyphenols by cinnamyl pyrophosphate:





As laboratory equivalents of these reactions they synthesized[10] both benzylstyrenes and neoflavanoids by Claisen rearrangement of resorcinol and pyrogallol cinnamyl ethers in boiling dimethylaniline, e.g. VI → VII + VIII.



KUMARI, MUKERJEE and SESHADRI[11], however, have questioned the validity of this analogy, since, in attempting to synthesize latifolin dimethyl ether by the Claisen rearrangement, they found that an ortho-methoxycinnamyl group does not apparently migrate. They proposed an alternative biogenetic scheme in which neoflavanoids and benzylstyrenes are formed by 2 *different* processes, the former by initial O-cinnamoylation of a phenol, rearrangement of the ester to a 4-aryl coumarin, and reduction of this to a dalbergione. In accord with OLLIS they considered benzylstyrenes are formed by C-cinnamylation of a phenolic unit and in support of this hypothesis they showed that 1,2,4-trimethoxybenzene condensed with ortho-methoxycinnamyl chloride in ether solution in the