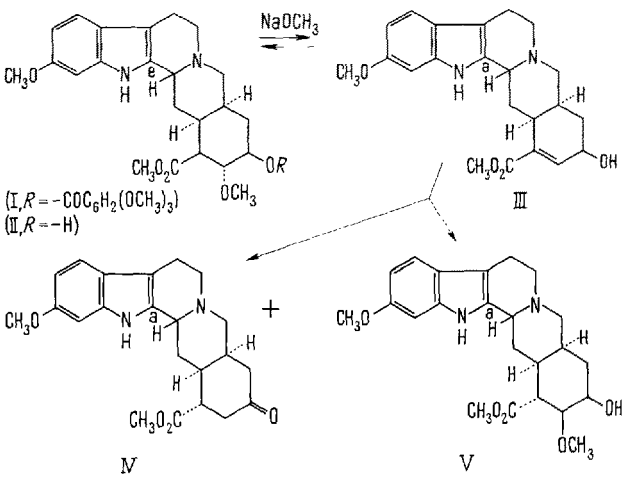
analysis was confirmed by the proton magnetic resonance spectrum[3] which showed only two methoxyl peaks (sharp) (τ values 6.18 and 6.20, respectively), each of intensity equivalent to 3 protons. A consideration of the mechanism proposed for the formation of methyl *neo*reserpate (V)[1b] provides a ready explanation for the production of both IV and V. Intermediate α,β-unsaturated γ-hydroxy ester III arises by a flip in conformation and reverse Michael-type elimination of methanol. Re-addition of methanol leads to V, while isomerization of the double bond followed by ketonization[4] leads to the 18-oxo derivative IV. The configuration at C-3 has remained β, as would be expected since 3-epialloyohimbanes are stable to base at this center[5], but is now axial. Confirming this, the infrared spectrum shows two distinct bands, at 2790 and 2850 cm^{-1}, characteristic for *trans*-quinolizidines[6]; the proton magnetic resonance spectrum[3] reveals no aliphatic peaks downfield from the two methoxyl peaks[7]; acid equilibration studies produce no new isomer[8]; and the substance readily forms a Δ^3-derivative (λ_{max} 376 mμ) upon controlled oxidation with mercuric acetate[9]. The 16-methoxycarbonyl function is assigned the thermodynamically more stable equatorial conformation because of the equilibrating conditions under which IV is formed.



All of these considerations are consonant with structure IV and lend additional experimental support for the mechanism proposed for the formation of methyl *neo*reserpate from reserpine. Details of these and other experiments will be published elsewhere.

Zusammenfassung. 11-Methoxy-18-oxo-3-epialloyohimban-16α-carbonsäureemethylester (IV) wurde nach Methanolyse des Reserpins isoliert. Wir nehmen an, dass der α,β-ungesättigte γ-Oxycarbonsäureester III die gemeinsame Zwischenstufe bei der Bildung von IV und Methyl-*Neo*reserpat darstellt.

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[3] Obtained with a Varian Model V-4300-B spectrometer operated at 56.4 mc using deuterated chloroform as solvent and tetramethylsilane as internal reference. We are indebted to Dr. J. LANCASTER of the Central Research Division, American Cyanamid Co., Stamford (Conn.), for this measurement.

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[8] H. B. MACPHILLAMY, C. F. HUEBNER, E. SCHLITTLER, A. F. ST. ANDRÉ, and P. R. ULSHAFER, J. Amer. chem. Soc. *77*, 4335 (1955).

[9] E. WENKERT and D. K. ROYCHAUDHURI, J. org. Chem. *21*, 1315 (1956).

Inhibition of Vaccinia Virus Multiplication by 2-Carboxymethylmercapto-4-amino-5-(*p*-chlorphenyl)-pyrimidine

A series of 5-arylpyrimidines were tested for their inhibitory activity for virus multiplication. Marked selective inhibition of vaccinia virus multiplication was found in the case of 2-carboxymethylmercapto-4-amino-5-(*p*-chlorphenyl-)pyrimidine (CACP) (Figure 1). A wide inhibitory zone of vaccinia virus plaque formation with a narrow zone of toxicity was produced by CACP in tissue culture (Figure 2).

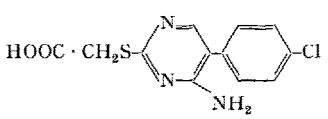

Fig. 1. 2-carboxymethylmercapto-4-amino-5-(*p*-chlorphenyl)-pyrimidine (CACP).

A more detailed evaluation of the inhibitory effect of CACP was carried out, using the membrane culture method which was described by TAMM[1–3] and partially modified by us[4]. The dependence of the inhibitory and toxic effects of CACP on its concentration in membrane cultures is shown in Figure 3. 75% virus inhibitory concentration, lowering vaccinia virus multiplication to 25% of the control, is 0.02 mg CACP/ml. The toxic concentration, which causes damage to the chorioallantoic membranes of a 2+ degree (according to TAMM[2]), is 0.3 mg CACP/ml.

The ratio of both values (i.e. toxic and virus inhibitory concentrations) is 15. Thus the selectivity of inhibition of

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vaccinia virus multiplication by CACP is approximately the same as that of 6-azauracil riboside[5]. That ratio for benzimidazole, the referential compound is 3, under the same conditions.

CACP, when tested by the plaque method, did not inhibit the multiplication of Newcastle disease virus (NDV) and Western equine encephalomyelitis virus (WEE).

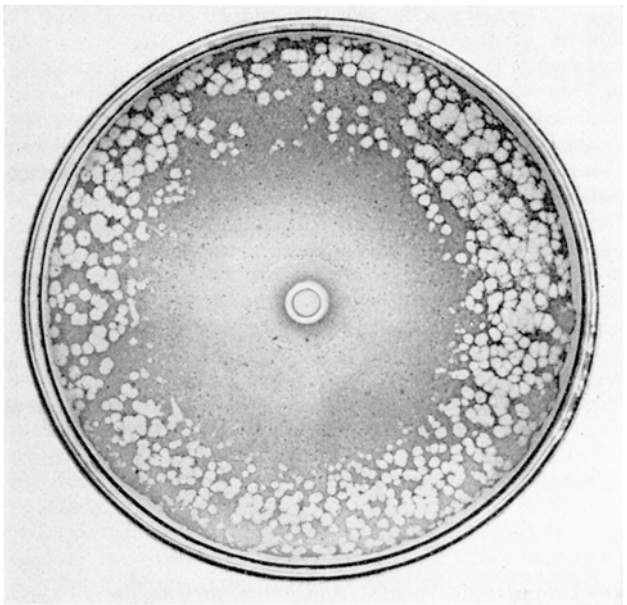

Fig. 2. Inhibitory effect of 2-carboxymethylmercapto-4-amino-5-(*p*-chlorphenyl)-pyrimidine. Monolayer of chick embryo cells was infected with 1000 plaque-forming units of vaccinia virus. After solidifying of the agar overlay, a glass cylinder was mounted in the gel. 0.05 ml of solution 10 mg CACP/ml was pipetted into the cylinder. 5 days after infection. Dish of 10 cm diameter.

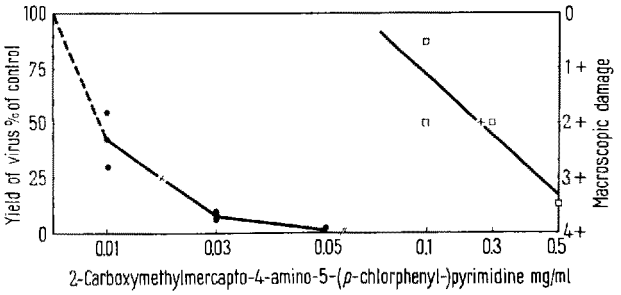

Fig. 3. Relationship between the concentration of CACP and the degree of inhibition of vaccinia virus multiplication and the extent of macroscopic damage to chorioallantoic membranes. • = yield of virus; ⌟ = macroscopic damage. The crosses × and + refer to the 75% virus inhibitory and 2+ toxic concentration respectively.

Zusammenfassung. Mit der Methode der Hemmung der Plaquebildung bei gleichzeitiger Diffusion der antiviralen Stoffe durch den Agar wurde eine hohe Selektivität des 2-Carboxymethylmercapto-4-amino-5(*p*-chlorphenyl)-pyrimidins für die Hemmung des Vaccinevirus festgestellt. Das Verhältnis der toxischen und der virushemmenden Konzentrationen wurde in den Membrankulturen =15 gefunden. Die Substanz war gegen andere Viren (NDV, WEE) unwirksam.

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Institute of Virology, Czechoslovak Academy of Sciences, Mlynská dolina, Bratislava, and Pharmaceutical and Biochemical Research Institute, Prague (Czechoslovakia), September 18, 1962.

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Activation of Amino Acids in the Liver in CCl_4 Intoxication

Recent findings on the fatty liver induced in rats by a wide variety of toxic substances show early damage in the extramitochondrial fractions of the cell[1–5].

With respect to CCl_4 poisoning, an hypothesis has been formulated regarding an inhibition of the hepatic triglyceride-secreting mechanism[6] probably related to an inhibition of lipoprotein formation. In this connection, a diminished incorporation of amino acids into the liver and plasma proteins[5,7] as well as into the plasma lipoproteins[8] has been reported.

The present work concerns a demonstrable inhibition of the first step of the protein synthesis, that is the amino acid activation, which occurs during the early stages of CCl_4 intoxication.

The assay was performed with the method of the amino-acyl hydroxamate formation by using the pH 5 enzyme obtained from liver according to Hoagland et al.[9]. Results of our experiments are shown in the Table.

The results presented in this communication suggest that the deficiency in the amino acid activation may account for the impairment of the protein and lipoprotein synthesis, as well as for the dramatic morphological changes, which are known to occur in the liver after CCl_4 administration. It is quite likely that such a deficiency may be set in the pathological sequence leading to either necrosis or steatosis, the latter being also related to the

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