tion became prominent. There was no effect on the initial flash-height peak when CoA was added initially; the total light emitted, however, was greater in the presence of CoA. AIRTH et al.[3] suggested the following explanation for this effect: CoA removes the inhibitory effect of oxyluciferin by reacting with it and forming the compound oxyluciferyl-CoA. This compound reacts with cysteine, as could be shown, and forms oxyluciferyl-cysteine. There was, however, no stimulation of the light emission by this secondary addition of cysteine to the reaction mixture. Moreover, no cysteine derivative of oxyluciferin could be formed in the absence of CoA, indicating that cysteine alone has no stimulatory effect.

Quite different from this are the results obtained in the Cypridina luciferase-luciferin system. Not only does the addition of cysteine alone increase the total light emitted but, also, it increases the initial flash-height peak. This stimulated light emission is directly proportional to the cysteine concentration. This indicates the possibility that cysteine is capable of reacting directly with the oxyluciferin compound or with any other inhibitor—whatever it is—of the Cypridina system. The increase in the initial flash-height peak indicates either a release of luciferase from small amounts of impurities still present in the enzyme solution or some kind of energy transfer. This latter reaction, the electron transition, seems more than likely. There was no indication that autoxidation is the process responsible for this effect. No autoxidation could be observed during the time the measurements were made[4].

Zusammenfassung. Cysteinlösung (Cystein-HCl gelöst in 0,1 *M* Phosphatpuffer) von verschiedenen Konzentrationen (0–4,65 *M*) wurde zu Luziferinlösung (gereinigtes kristallines Luziferin gelöst in Methanol, 0,1 mg/ml) oder zu praktisch reiner, gepufferter Luziferaselösung (0,1 mg/ml) zugegeben. Mit zunehmenden Cystein-Konzentrationen ergibt sich eine Zunahme in der Höhe der Reaktionskurve.

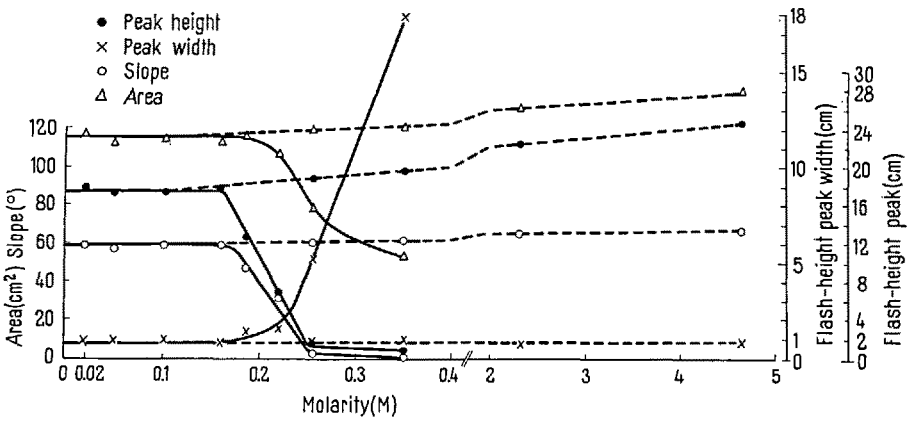

Fig. 2. Influence of cysteine concentration on the Cypridina luciferase-luciferin system. 25 λ of cysteine were added to the same quantity of luciferase in either 25 λ (solid line) or 10 ml (dotted line) of solution.

W. LOHMANN, C. F. FOWLER, and W. H. PERKINS

Departments of Radiology and Medicine, University of Arkansas Medical Center; and Radioisotope Service, VA Hospital, Little Rock (Arkansas, U.S.A.),
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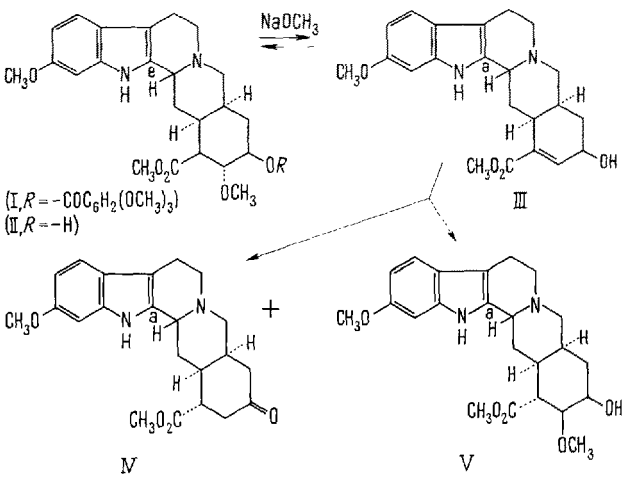
Methyl 11-Methoxy-18-oxo-3-epialloyohimban-16α-carboxylate, a New Keto Ester Derived from Reserpine

Recent reports on the chemistry of methyl *neo*reserpate (V)[1] prompt us to describe some experiences with preparation of this compound. Our investigations lend additional experimental support for the mechanism proposed for formation of V from reserpine (I)[1b]. Extended reaction times (refluxing for 64 h with sodium methoxide) in the methanolysis of reserpine to methyl reserpate (II) resulted in the formation of the more stable methyl *neo*-reserpate (V)[1a]. We have found that an additional product is formed when the reaction is carried out in a glass-lined bomb at 100° for 6 h. Although a small amount of methyl *neo*reserpate was recovered from accumulated mother liquors by alumina chromatography, the major product, formulated as methyl 11-methoxy-18-oxo-3-epialloyohimban-16α-carboxylate (IV), appeared to be a keto ester from its infrared spectrum ($\nu_{\text{Nujol}}^{\text{max}}$ 1730 and 1700 cm^{-1}). This colorless crystalline substance, m.p. 114.5–117.5°, $[\alpha]_D^{25}+69°$ (*c*, 0.49 in pyridine) (Found: C, 67.0; H, 7.08; N, 7.45; $(O)CH_3$, 7.78; H_2O, 4.90. $C_{22}H_{26}N_2O_4 \cdot 3/4\,H_2O$ requires C, 66.7; H, 6.99; N, 7.07; $2(O)CH_3$, 7.58; H_2O, 3.41), gave a bright yellow crystalline 2,4-dinitrophenylhydrazone, m.p. 194–204°, $\nu_{\text{max}}^{\text{Nujol}}$ 1732 cm^{-1}, $\lambda_{\text{CHCl}_3}^{\text{max}}$ 360 mμ[2]. The loss of methoxyl suggested by the

[1] (a) W. E. ROSEN and J. M. O'CONNOR, J. org. Chem. *26*, 3051 (1961). – (b) W. E. ROSEN and H. SHEPPARD, J. Amer. chem. Soc. *83*, 4240 (1961). – (c) W. E. ROSEN and J. N. SHOOLERY, J. Amer. chem. Soc. *83*, 4816 (1961).

[2] For visible spectra of other saturated ketone dinitrophenylhydrazones see C. DJERASSI and E. RYAN, J. Amer. chem. Soc. *71*, 1000 (1949). – J. P. PHILLIPS, J. org. Chem. *27*, 1443 (1962).

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.

L. A. MITSCHER, J. K. PAUL, and L. GOLDMAN

Organic Chemical Research Section, Lederle Laboratories, American Cyanamid Company, Pearl River (New York, U.S.A.), December 10, 1962.

[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.

[4] A mechanistically similar isomerization was reported by C. P. BALANT and M. EHRENSTEIN, J. org. Chem. *17*, 1587 (1952).

[5] P. E. ALDRICH, P. A. DIASSI, D. F. DICKEL, C. M. DYLION, P. D. HANCE, C. F. HUEBNER, B. KORZUN, M. E. KUEHNE, L. H. LIU, H. B. MACPHILLAMY, E. W. ROBB, D. K. ROYCHAUDHURI, E. SCHLITTLER, A. F. ST. ANDRÉ, E. E. VAN TAMELYN, F. L. WEISENBORN, E. WENKERT, and O. WINTERSTEINER, J. Amer. chem. Soc. *81*, 2484 (1959).

[6] F. BOHLMANN, Ber. dtsch. chem. Ges. *91*, 2157 (1958). – E. WENKERT and D. K. ROYCHAUDHURI, J. Amer. chem. Soc. *78*, 6417 (1956).

[7] C-3 equatorial proton signals are observed at 5.67τ in pseudoyohimbine (J. D. ALBRIGHT, L. A. MITSCHER, and L. GOLDMAN, J. org. Chem. *28*, 38 (1963)) and at 5.56τ in reserpine and methyl reserpate[1c] whereas C-3 axial proton signals are observed at higher field.

[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).

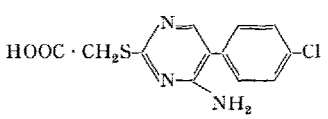


Fig. 1. 2-carboxymethylmercapto-4-amino-5-(*p*-chlorphenyl)-pyrimidine (CACP).

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

[1] I. TAMM, K. FOLKERS, and F. L. HORSFALL JR., J. exp. Med. *98*, 229 (1953).

[2] I. TAMM, J. Bact. *72*, 42 (1956).

[3] I. TAMM and J. R. OVERMAN, Virology *3*, 185 (1957).

[4] B. RADA and D. BLAŠKOVIČ, Acta Virol. *5*, 308 (1961).