non-bonded interactions that are not produced in β-equatorially substituted compounds. Hence the results for compounds IIa–d and III. The ketone (V) is known to be *cis*[4], thus ketal formation does not introduce unfavourable 1,3-interactions. The ketone (IV) is a *cis-trans* mixture in which the latter form predominates (from results of the isomeric composition of derived 4-phenyl-4-piperidinols)[5] and the ketal, isolated in small yield, most likely derives from the *cis*-component. Ketal formation with the ketone (VI) necessarily introduces new 1,3-diaxial interactions and is thus unfavoured. Facile acid-catalysed ketal formation in 4-piperidones is probably a result of carbonyl carbon being activated towards nucleophilic attack by the electronic influence of the protonated nitrogen atom. This influence is reflected in the lower carbonyl absorption frequencies of 4-piperidones in comparison with those of their salts [e.g. 1-(2-phenethyl)-4-piperidone base, 1717 cm^{-1}; HCl salt, 1736 cm^{-1}][12].

The related phenomenon of hydrate formation in 1-methyl-4-piperidone has been reported by LYLE, ADEL, and LYLE[6].

Zusammenfassung. Die Reaktion gewisser heterocyclischer Ketone mit Äthanol in Gegenwart von Säure ist abhängig von der Ringgrösse und wird plötzlich durch die Wechselwirkung von 1,2-*cis*- oder 1,3-diaxalen Substituenten beeinflusst.

A. F. CASY

School of Pharmacy, Chelsea College of Science and Technology (London), February 17, 1964.

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Inhibitory Activity of Benzoyl Hydrazides and Hydrazine on the Growth of Influenza Virus in Chick Embryo Lung Tissue Culture[1]

Hydrazine and its derivatives have occasionally been found to be effective chemotherapeutic agents against various microbial agents[2–8], but with the exception of the inhibition of Theiler's virus in tissue culture by 1-hydrazinophthalazine (Apresoline)[9], there is no report of effective viral antagonism by hydrazine derivatives.

In the course of testing the antiviral properties of analogs of amino acids and amino acid precursors[10,11], anthranilic acid hydrazide was found to inhibit the growth of influenza virus in chick embryo lung tissue culture. This report deals with the study of the antiviral characteristics of hydrazides, particularly anthranilic acid hydrazide.

Materials and methods. Hydrazine was purchased from the Eastman Kodak Co., Rochester, N.Y., and the five effective hydrazide compounds were kindly supplied by Hoffmann-La Roche, Nutley, N.J. The technique of the use of chick embryo lung tissue culture in the testing of the inhibition of growth of the influenza virus by drugs was described previously[10]. Briefly, influenza A virus, WS strain, aliquots of the drug, and finely dispersed embryonic chick lung were put in rubber-stoppered tubes. After incubation at 36°C for 44 h in a roller drum, the tubes were examined under 100× magnification for drug toxicity to the chick cells. Tubes showing normal tissue growth were tested for the growth of virus by means of the hemagglutination technique. The criterion for positive inhibition of virus by a compound was an eightfold or greater reduction of the hemagglutination end point.

Results. In the course of testing some 900 metabolically active compounds[11], anthranilic acid hydrazide, benzoic acid hydrazide, 2-methoxybenzoic acid hydrazide, *m*-nitrobenzoic acid hydrazide, salicylic acid hydrazide, as well as hydrazine itself, were found to inhibit effectively the growth of influenza virus in tissue culture (Table), while 16 other hydrazine derivatives were found to be ineffective or only partially effective.

In order to determine the effect of anthranilic acid hydrazide upon the growth of virus chick embryo lung tissue culture tubes were inoculated with 10^3 EID_{50}[12] of influenza virus. An inoculum of 0.25 mg of anthranilic acid hydrazide per ml of medium was subsequently added to tubes at intervals from 0 to 8 h after virus administration. The virus titer in these tubes was determined after 24 h of incubation. In addition the virus titers in the tube receiving the compound at zero h and

[1] This investigation was supported by a contract with the Office of Naval Research.
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[12] EID_{50} = the dose of virus which will infect 50% of the eggs inoculated.

the drug-free control tube were determined after 30 h of incubation. The results are shown in the Figure. Anthranilic acid hydrazide inhibited virus growth when added within 6, but not 8, h after virus inoculation. After 30 h incubation there was a greater than four log difference between the tube receiving only virus and the tube receiving drug and virus simultaneously.

To determine whether virus inhibition was due to a direct virucidal effect of the hydrazide, 4 mg of anthranilic acid hydrazide per ml of medium were added to a tube containing influenza virus. The tubes were incubated at pH 7.0 at 37° C. After intervals of 0, 1, 2, 4, 6, and 8 h, aliquots of fluid were removed for virus titration in eggs. There was no significant virucidal activity.

Anthranilic acid hydrazide inhibited the growth of influenza B (Lee strain) and influenza A virus in chick embryo lung tissue culture to the same extent.

Because of the close association of anthranilic acid and benzoyl acids with the formation of aromatic amino acids, nicotinic acid and other metabolites, an attempt was made to antagonize the inhibitory capacities of anthranilic acid hydrazide by various biologically active compounds. Neither anthranilic acid, quinic acid, kynurenic acid, tyrosine, phenylalanine, *p*-aminobenzoic acid, aniline, tryptophan, indole, nor nicotinic acid reversed the antiviral activity, suggesting that the inhibitory property may be associated with the hydrazide moiety.

4 mg of anthranilic acid hydrazide inoculated into the allantois of each of 6 fertile hens' eggs failed to inhibit the growth of influenza virus. Anthranilic acid hydrazide was non-toxic to mice at a concentration of 0.5% in the diet. It also failed to alter the course of infection in mice inoculated intranasally with influenza virus.

Discussion. Hydrazine in free or dissociable form appears to be an effective inhibitor of influenza virus growth in tissue culture, even when added up to 6 h after the virus. There was no alteration of activity when the hydrazine was bound as a hydrazide to a benzyl ring other than to form relatively insoluble (and under conditions of our tests, ineffective) compounds when other groups were added in the *meta* or *para* position[11].

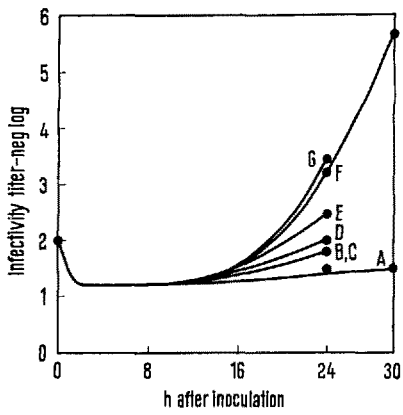
The mechanism of action of virus inhibition of these 6 compounds is not understood. Their consistent therapeutic index makes it appear that this mechanism is identical in each of the 5 inhibitors. It is suggested that once the benzoyl hydrazides penetrate the cell the compounds are hydrolyzed to benzoic acid plus hydrazine, and the latter compound, being an active reducing agent, may then serve as a virus inhibitor in one or more ways to interfere with cell metabolism. Others have found that hydrazine can replace NH_3 in the synthesis of glutamine[13], that it may combine with the keto groups of sodium pyruvate and ketoglutarate[14], that hydrazides act as amine oxidase inhibitors[15], and that phenylhydrazine inhibited both the deamination and the desulfuration of cysteine[2]. Hydrazines have also been reported to inhibit enzymatic processes by acting as a chelating agent of heavy metals[16].

It is of interest that the molar activity of hydrazine necessary for virus inhibition is about 20 times that of the benzoyl hydrazides. This is consistent with the premise that the hydrazides must enter the cell to be hydrolyzed prior to their activation so that an excess of benzoyl hydrazide is required for effective virus inhibition.

Hydrazides effective in inhibiting the growth of influenza A virus in tissue culture

Compound	MTC[a] (mg/ml)	MCI[b] (mg/ml)	TI[c]
(1) Hydrazine	0.016	0.002	8
(2) Anthranilic acid hydrazide	2.0	0.25	8
(3) Benzoic acid hydrazide	2.0	0.25	8
(4) 2-Methoxybenzoic acid hydrazide	1.0	0.12	8
(5) *m*-Nitrobenzoic acid hydrazide	0.5[d]	0.06	8
(6) Salicylic acid hydrazide	2.0	0.25	8

[a] Maximum tolerated concentration by tissue. [b] Minimum concentration inhibitory to virus. [c] Therapeutic index (a/b). [d] Ml of a saturated solution of compound per ml of medium.



Effect of delayed addition of 0.25 mg anthranilic acid hydrazide per ml upon growth of influenza virus in chick embryo lung tissue culture. Titers measured in eggs. Drug added (A) same time as virus; (B) 1 h; (C) 2 h; (D) 4 h; (E) 6 h; (G) 8 h after virus; (F) no drug added. Titers are to the $\log_{10}$ of 0.1 ml culture fluids.

Zusammenfassung. Hydrazin, wie auch verschiedene Benzoylhydrazide, hemmen das Wachstum des Influenzavirus in Kulturen embryonalen Hühnerlungengewebes. Anthranilsäurehydrazid konnte die Virusvermehrung bis 6 h nach Viruszugabe hemmen.

W. D. Kundin[17,18], Mary Louise Robbins, and P. K. Smith[19]

Departments of Pharmacology and of Microbiology, The George Washington University School of Medicine, Washington (D.C. USA), March 23, 1964.

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