ANTIMICROBIAL ACTIVITY OF PATULIN DERIVATIVES: A PRELIMINARY REPORT

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Preliminary results are reported of antimicrobial assays conducted with chemically synthesized derivatives of patulin, a mycotoxin produced by several species of *Penicillia* and *Aspergilli*. Patulin (Fig. 1) has been shown to be an antibiotic[1] and a carcinogen[2]; it is considered too toxic for humans or animals[3] and is inhibitory to plants[4]. It is an α,β-unsaturated γ-lactone with two conjugated double bonds and a very reactive hemiacetal group that racemizes rapidly in aqueous media, precluding isolation of the (+) and (−) optical isomers.

TANABE and SUZUKI[5] reduced the hemiacetal group of patulin to form ascladiol, a compound reported to be 25% as toxic as the parent compound although it has the same unsaturated bonds and γ-lactone structure. No details of their toxicity tests were given. LIEU and BULLERMAN[6] observed decreased activity against *Bacillus subtilis*, brine shrimp larvae, and chick egg embryos when patulin reacted with sulfhydryl (–SH) groups of cysteine or glutathione. Although postulated to be the result of MICHAEL addition of sulfhydryl to the double bonds of patulin, such groups can also react with the hemiacetal moiety, and this may explain the decrease in toxicity of the reaction products. Some beneficial uses of patulin are mentioned in the report by SINGH[4] of the work of OOSTERHUIS, who used patulin for curing clinical dermatomycoses, and in a patent issued to VERMEULEN and LE DRUT[7], who prepared patulin thiosemicarbazone and found it to be a bacteriolytic agent against KOCH bacilli without the expected bacteriostatic activity of patulin against Gram-positive and Gram-negative bacilli. Because of the solubility of patulin thiosemicarbazone in lipids, the derivative readily resorbed *via* the lymphatic passages and gave favorable results in the treatment of pulmonary and several other forms of tuberculosis.

The lack of information on the biological activity of patulin derivatives, as well as the work cited above, led us to prepare thirteen compounds for evaluation of their toxicity and teratogenicity relative to patulin. The following derivatives (Fig. 1), prepared by established procedures, were characterized on the basis of their physical constants and spectral properties: lactone **2**[8], acetate **3** and benzoate **4**[9], oximes **5**, **6**, **7** and **8**[10], semicarbazone **9** and thiosemicarbazone **10**[7] hydrazones **11** and **12**[9], dimethone anhydride **13**[9], and octahydroxanthene **14**[11]. Preparation of derivatives utilized the free hydroxyl group of patulin to give a lactone (oxidation of hydroxyl) or esters (acylation of hydroxyl). In solution, the hemiacetal reacted as an aldehyde with appropriate reactants to give the other derivatives reported here. As a preliminary step, a series of disc assays was run and the results are reported here. These assays may aid in the discovery of new products of pharmacological interest; subsequent toxicity tests to be conducted later will utilize egg embryos and small animals to help determine the role in living systems of the reactive moieties of patulin, such as the unsaturated bonds and hemiacetal group.

The derivatives were tested at a concentration of 100 μg/paper disc for activity against six microorganisms. This concentration assumes an average take-up of 0.15 ml of test solution per disc, from a solution containing 2 mg test compound in 3 ml of solvent. All compounds were dissolved in methanol (non-inhibitory solvent) except patulin and the free oximes, which were soluble in water. Seeded agar plates were prepared as described by PRIDHAM *et al.*[12]. The microorganisms used were: *Bacillus subtilis* NRRL B-765, *Micrococcus luteus* NRRL B-1018, *Escherichia coli* NRRL B-766, *Saccharomyces cerevisiae* NRRL Y-139, *Candida albicans* NRRL Y-477, and *Mucor ramannianus* NRRL 1839, representing, respectively, two Gram-positive bacteria, one Gram-negative bacterium, two yeasts and a mold. Thus, patulin derivatives were tested for their ability to inhibit spore germi-

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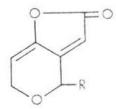
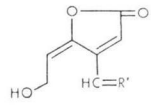
Fig. 1. Structures of derivatives.



Patulin
(Hemiacetal form)



Patulin
(Aldehyde form)

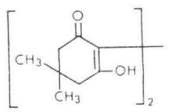
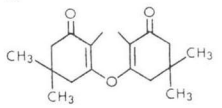
Compound No.	Patulin derivative	R	R′
1	Patulin	–OH	=O
2	Lactone	=O	—
3	Acetate	$-O-COCH_3$	—
4	Benzoate	$-O-COC_6H_5$	—
5	*syn*-Oxime	—	$=N^{OH}$
6	*anti*-Oxime	—	$=N_{OH}$
7	*syn*-O-Methyloxime	—	$=N^{OCH_3}$
8	*anti*-O-Methyloxime	—	$=N_{OCH_3}$
9	Semicarbazone	—	$=N-NH-CONH_2$
10	Thiosemicarbazone	—	$=N-NH-CSNH_2$
11	Phenylhydrazone	—	$=N-NH-C_6H_5$
12	2,4-Dinitrophenylhydrazone	—	$=N-NH-C_6H_3(NO_2)_2-2,4$
13	Dimethone anhydride	—	
14	Octahydroxanthene	—	

Table 1. Test organisms and antibiotic activity of patulin derivatives.

Patulin derivative	Diameter (mm) of clear zone					
	B. subtilis	*M. luteus*	*E. coli*	*S. cerevisiae*	*C. albicans*	*M. ramannianus*
1 Patulin	42.0[a]	32.0[b]	31.6	0	0	20.0
2 Lactone	23.0	20.5[c]	21.5	17.0	14.5	21.0
3 Acetate	36.0	29.2	30.0	15.5	tr	35.0
4 Benzoate	31.3	22.8	tr	20.5	16.5	39.0
5 *syn*-Oxime	40.0	31.2	31.0	0	0	17.5
6 *anti*-Oxime	41.0	31.6	30.1	0	0	tr?
7 *syn*-O-Methyloxime	38.0	32.4	27.8	tr?	0	40.0
8 *anti*-O-Methyloxime	38.0	35.6	33.3	15.3	15.5	39.0
9 Semicarbazone	0	0	20.5	0	0	0
10 Thiosemicarbazone	tr	16.5	17.5	0	0	0
11 Phenylhydrazone	24.0	21.5	tr?	tr?	0	26.0
12 2,4-Dinitrophenylhydrazone	22.1	22.0	tr?	tr?	0	0
13 Dimethone anhydride	0	0	0	0	0	0
14 Octahydroxanthene	0	0	0	0	0	0

[a] Ring at 35.5 mm. [b] Ring at 28.0 mm. [c] Ring at 14.8 mm.

nation (*B. subtilis*), vegetative growth (*M. luteus*, *E. coli*, *S. cerevisiae*, and *C. albicans*), and sporangiospore germination (*M. ramannianus*). Although assay plates containing *B. subtilis* and *M. ramannianus* were prepared with spore suspensions, it is possible that germination occurred and that subsequent growth was inhibited. The results are presented in Table 1.

The assay results indicate great variation in antimicrobial activity of patulin and its derivatives among the six test organisms. Of particular interest are patulin lactone (Fig. 1, number **2**) and the *anti*-O-methyloxime, which are unique in being inhibitory to all test organisms. The former compound has both an α,β-unsaturated γ-lactone and a β,γ-unsaturated δ-lactone. Dimedon, used to prepare patulin dimethone anhydride and patulin octahydroxanthene, showed the same lack of activity as the derivatives. This lack of response may be due in part to the molecular size and/or configuration of the derivative, thus preventing any reaction with those molecular species essential for growth.

Patulin, the lactone, and the *syn*-oxime showed antifungal activity against *Mucor ramannianus* 1839, but larger clear zones up to twice the diameter were produced by esterified patulin (acetate, benzoate), the phenylhydrazone, and the O-methyloximes. Although these latter derivatives have the –C=N-linkage, the semicarbazone and thiosemicarbazone were inactive against this fungus; thus the antifungal activity does not seem to depend upon this linkage *per se*. Also, patulin 2,4-dinitrophenylhydrazone showed no antifungal activity. The semicarbazone showed moderate inhibition of growth of the Gram-negative *E. coli*, but not against any other organism; the thiosemicarbazone was active against both *E. coli* and *M. luteus*, a Gram-positive bacterium.

We conclude that addition of sulfhydryl groups to the unsaturated bonds of patulin may be only one of several biological reactions whereby patulin exerts its antimicrobial activity. Chemical derivatives of patulin produce a variety of biological responses among different microbial species. Certain derivatives are effective inhibitors of spore and sporangiospore germination as well as of vegetative growth, whereas others are totally ineffective. The inhibition of growth in certain instances may be a function of the derivatizing group attached to patulin, rather than of the unsaturated γ-lactone or the conjugated double bonds. Our data indicate the hemiacetal group is involved in patulin toxicity, although the results vary according to the type of derivative tested. Other work in progress to ascertain the toxicity and teratogenicity of these compounds against chick egg embryos and small animals will be reported later.

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