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Lupulon—An Antibiotic Extracted from the Strobiles of *Humulus lupulus.**†

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Lupulon is a nearly white, crystalline substance obtained by fractionating the resins extracted with methanol and other solvents from the strobiles of the hop *Humulus lupulus.*

According to Taylor and Millidge[1] lupulon possesses the following properties: It has a melting point of 92°C, is unsaturated, adds bromine, decolorizes permanganate, and has the properties of a keto-enol.

Walker and Parker[2] reported that the hop resins exhibited antibacterial properties. Shimwell[3] found that lupulon inhibited the growth of Gram-positive bacteria but had no effect on Gram-negative organisms.

The present communication is concerned with a study of the bacteriologic and mycologic properties of lupulon *in vitro* and its use in the treatment of bacterial infections in mice.

Materials and methods. A solution of lupulon was prepared by dissolving one gram in 25 ml of propylene glycol, heating slightly, then adding sufficient distilled water to make a 1:500 opalescent emulsion of the resinous antibiotic.

The bactericidal (with the exception of *Mycobacterium tuberculosis*) and fungicidal spectra were determined by the penicylinder method using a medium having the following composition: Sodium chloride (NaCl), 5 g; beef extract (Bacto), 5 g; peptone (Bacto), 10 g; agar (Bacto), 20 g; and distilled water, to make 1000 ml. The medium was adjusted

TABLE I.
Effect of Lupulon on a Number of Bacteria by the Penicylinder Method.

Gram-positive bacteria 1:10,000 dilution of lupulon	Results	Gram-negative bacteria 1:500 dilution of lupulon	Results
Micrococcus lysodeikticus	—	*Aerobacter aerogenes*	+
" *pyogenes* var. *aureus*	—	*Alcaligenes faecalis*	+
" *ureae*	—	" *viscosus*	+
Bacillus anthracis	—	*Escherichia coli*	+
" *megatherium*	—	" *coli* var. *communior*	+
" *subtilis*	—	*Klebsiella pneumoniae*	+
Gaffkya tetragena	—	*Proteus vulgaris*	+
Rhodococcus roseus	—	*Pseudomonas aeruginosa*	+
Sarcina lutea	—	" *synxantha*	+
" *ureae*	—	*Salmonella enteritidis*	+
Streptococcus faecalis	—	" *paratyphi A*	+
" *lactis*	—	" *schottmuelleri*	+
		" *typhosa*	+
		Serratia marcescens	+
		Shigella ambigua	+
		" *dysenteriae*	+
		" *paradysenteriae*	+
		" *sonnei*	+

+ = growth; — = no growth.

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† The lupulon used in these experiments was kindly supplied by the Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif.

1 Taylor, T. W. J., and Millidge, A. F., Richter's Organic Chemistry, Vol. II, Nordeman Publishing Co., Inc., New York, 1939.

2 Walker, T. K., and Parker, A., *J. Inst. Brew.*, 1937, **34**, 17.

3 Shimwell, J. L., *J. Inst. Brew.*, 1937, **34**, 111, 191.

TABLE II.
Effect of Lupulon on a Group of Higher Organisms by the Penicylinder Method.

1:500 dilution of lupulon	Results
Aspergillus flavus	+
" *niger*	+
Candida albicans	+
Cryptococcus neoformans	+
Discomyces mexicanus	+
Epicoccum nigrum	+
Microsporum lanosum	+
Nocardia asteroides	+
" *madurae*	+
Penicillium glabrum	+
Rhizopus nigricans	+
Streptomyces pelletieri	+
Trichoderma köningii	+
Trichophyton gypseum	+

+ = growth.

to pH 7.0 and sterilized at 15 lb pressure for 30 minutes.

Proskauer and Beck's medium was used for the growth of *M. tuberculosis*. This is a liquid medium having the following composition: Asparagine, 5 g; potassium acid phosphate (KH_2PO_4), 5 g; potassium sulfate (K_2SO_4), 0.6 g; magnesium citrate soluble ($MgHC_6H_5O_7 \cdot 5H_2O$), 2.5 g; glycerol, 25 g; and distilled water, to make 1000 ml.

Results. The action of lupulon on a number of Gram-positive and Gram-negative bacteria by the penicylinder method is given in Table I. It may be seen that a 1:10,000 dilution of the antibiotic was effective against Gram-positive bacteria but displayed little or no activity against Gram-negative organisms even when used in a concentration as high as 1:500.

In Table II are given the results of lupulon on a group of higher organisms. The antibiotic produced no apparent effect when used in a 1:500 dilution.

Lupulon was tested against several organisms in the presence of 10% horse serum‡ (Table III). The results show that lupulon exhibited no antibiotic effect when tested in the presence of this agent.

Eight mice were injected with 0.1 cc of a virulent broth culture of *Streptococcus pyogenes*. Four of the mice were treated with lupulon and 4 were used as controls. Doses of 2 mg of the antibiotic injected intraperitoneally every 3 hours failed to produce any demonstrable effect on the outcome of the infection. All animals were dead after 24 hours. It may be concluded that the antibiotic was completely inactivated *in vivo*.

According to Taylor and Millidge[1] lupulon has the structural formula shown in (A), Fig. 1. Lupulon may exist in any of the 3 forms shown but forms (B) and (C) probably predominate because they contain more highly conjugated systems and hence have more resonance of stabilization than (A).

Lupulon is slowly inactivated when in solution. The effect of a freshly prepared and an old solution of lupulon on *M. tuberculosis* H37Rv is shown in Table IV. A freshly prepared solution is considerably more effective than one 10 days old.

Inactivation on standing is compatible with the general instability of the molecule. The side chain in position 5 contains an allylic

‡ The horse serum used in these experiments was kindly supplied by the Cutter Laboratories, Berkeley, Calif.

TABLE III.
Effect of Lupulon on *Micrococcus pyogenes* var. *aureus* and *Mycobacterium tuberculosis* H37Rv in the Presence and Absence of Horse Serum.

Dilution of lupulon	*M. pyogenes* var. *aureus*		Dilution of lupulon	*M. tuberculosis*	
	With 10% serum	Without serum		With 10% serum	Without serum
1:10 × 1000	+	—	1:10 × 1000	+	—
1:20 "	+	—	1:20 "	+	—
1:25 "	+	—	1:60 "	+	—
1:33 "	+	—	1:100 "	+	—
1:60 "	+	+	1:160 "	+	—
1:100 "	+	+	1:200 "	+	—
1:200 "	+	+	1:300 "	+	+
1:300 "	+	+	1:400 "	+	+
Control	+	+	Control	+	+

+ = growth; — = no growth.

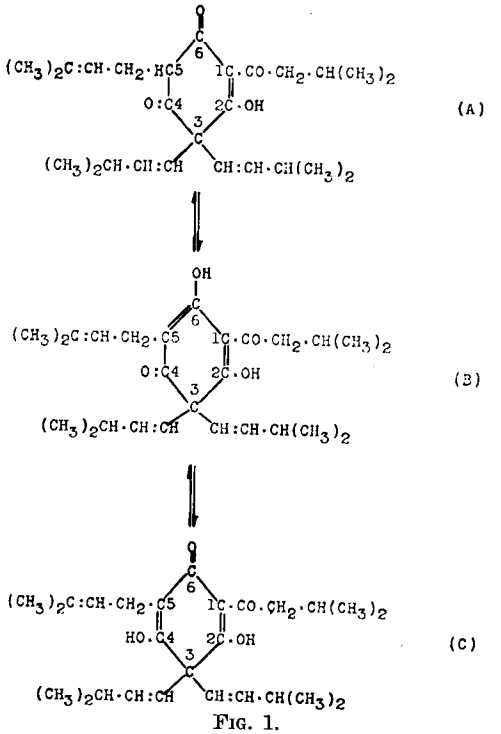

FIG. 1.

Lupulon may exist in any of the 3 forms shown above but probably (B) and (C) predominate.

methylene group in an extremely unstable arrangement. The double bonds in the 2 side chains in position 3 are susceptible to oxidation by air. The semi-aromatic system itself is, in general, an unstable configuration.

One would expect the hydroxyl in position 6 of tautomer (B) or the hydroxyl group in position 4 of tautomer (C) to acetylate under normal conditions. The hydroxyl in position 2 could be acetylated only with difficulty because of chelation with the carbonyl group on the one hand, and the hindrance due to the alkyl group in position 3 on the other.

Summary and Conclusions. Lupulon, an antibiotic extracted from hop resins, was found to be effective against Gram-positive bacteria *in vitro* but inactive against Gram-negative organisms. The antibiotic had no demonstrable effect on a number of pathogenic and nonpathogenic molds and actinomycetes.

Lupulon displayed no activity in the presence of horse serum. In the absence of serum the antibiotic exhibited its greatest activity against *M. tuberculosis* H37Rv. This may have been due to the fact that the tubercle bacillus was cultivated in a mineral medium with only an appreciable amount of interfering organic matter whereas the other organisms were grown in a medium containing peptone. The addition of serum to both media completely inactivated the antibiotic.

Lupulon is slowly inactivated in the presence of air and more rapidly in the presence of serum. Because of its inactivation by serum, the antibiotic is not suitable for clinical administration.

TABLE IV.

Effect of a Freshly Prepared and an Old Solution of Lupulon on *Mycobacterium tuberculosis* H37Rv.

Dilution of lupulon	Freshly prepared solution	Solution 10 days old
1:60 × 1000	—	—
1:80 "	—	—
1:100 "	—	+
1:140 "	—	+
1:180 "	—	+
1:200 "	—	+
1:300 "	—	+
Control	+	+

+ = growth; — = no growth.