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*ANTIBIOTIC ACTIVITY OF LICHENS**

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The production by an organism of organic substances inimical to the life processes of other organisms and inhibiting their growth is known as antibiosis. The phenomenon of antibiosis has attracted wide-spread attention in recent years chiefly because of the remarkable chemotherapeutic effects of penicillin, a product of the mold *Penicillium*. The anabolic products of many kinds of bacteria,[1] molds,[2] and algae,[3] and substances elaborated by certain species of flowering plants[4] can be readily demonstrated to have antibiotic properties when small amounts of the active materials are tested on microörganisms with suitable assay procedures. In view of the reported antibacterial activity of the green alga *Chlorella* and the many antagonistic substances now known to be produced by numerous kinds of fungi, the lichens seemed to offer favorable material for antibiotic investigations, inasmuch as the bodies of these plants are comprised of mixtures of algae and fungi. Accordingly we have tested a considerable number of lichens for antagonistic action against some common species of bacteria, and offer herewith a preliminary report concerning some of our observations.

Methods.—The lichens were brought in fresh condition to the laboratory, placed in shallow pans, and moistened with water for the purpose of allowing them to become physiologically more active. After a few hours of exposure to sunlight, samples of the moist lichens were separated from the substrata with a razor blade and forceps. Extracts were made by grinding, with a glass mortar and pestle, 100 mg. of lichen in 1 ml. of phosphate buffer solution adjusted to pH 7.4. The final aqueous extract was approximately pH 7.0. Extractions were carried out at room temperature and the samples were stored at 1°C. for a short time until the materials could be assayed. No attempt was made to clarify the extracts in these preliminary tests. The aqueous suspensions were assayed against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* or other bacteria by means

of the cylinder plate procedure.[5] As is well known to workers in this field, the method involves the use of agar plate cultures of bacteria upon which are placed small glass cups to which are added the assay liquids. After a period of incubation, zones of bacterial growth-inhibition subjacent to the cups are found to vary in diameter depending upon the potency of the test solutions which have diffused out of the cups into the agar. In these preliminary tests with aqueous suspensions the diameter of the zones of inhibition ranged up to 22 mm.

TABLE 1

ANTIBIOTIC ACTIVITY OF EXTRACTS OF LICHENS IN PHOSPHATE BUFFER TESTED AGAINST THREE BACTERIA. + = INHIBITED; − = NOT INHIBITED

SPECIES OF LICHEN	*S. aureus*	*E. coli*	*B. subtilis*
C. caespiticia	+	−	+
C. capitata	+	−	+
C. coniocraea	+	−	+
C. cristatella	+	−	+
C. glauca	+	−	+
C. Grayi	+	−	+
C. nemoxyna	+	−	+
C. squamosa	+	−	+
C. strepsilis	+	−	+
Parmelia physodes	+	−	+
Physcia sp.	+	−	+
Ramalina sp.	+	−	+
Sterocaulon paschale	+	−	+
C. incrassata	−	−	+
C. atlantica	−	−	+
C. subtenuis	−	−	+
C. caroliniana	−	−	+
C. uncialis	−	−	+
Lepra sp.?	−	−	+
Parmelia conspersa	−	−	+
Peltigera sp.	−	−	+
C. furcata	+	−	−
C. papillaria	+	−	−
Umbilicaria papulosa	+	−	−

Further experiments were performed with selected species of lichens for the purpose of determining whether the active compounds could be extracted with solvents, such as ether, chloroform and ethyl alcohol. The moist lichen materials were ground in a glass mortar, using 100 mg. of lichen to approximately 1 ml. of solvent. The suspensions were then centrifuged, and the clear solutions were pipetted into small flasks which could be attached to a vacuum distillation apparatus for removing the solvents at low temperature. The residual matter was taken up in aqueous phosphate buffer at pH 7.4, and after mixing with a glass rod the material was

tested in the cup plates. Incubation of the test plates was allowed to proceed for 12 hours at 37°C.

Results.—Of the 42 species of lichens tested thus far, 27 have been found active against either *S. aureus* or *B. subtilis.* Some of these are listed in table 1. In addition to those given in the table, *Cladonia cryptochlorophaea, C. pyxidata* and *Dermatocarpon miniatum* were found to inhibit *S. aureus,* but were not tested with *B. subtilis.* None of the 42 lichens which were tested shows any activity against *E. coli.* Slight inhibition against *Alcaligenes fecalis* was noted for the lichen species *C. glauca* and *Parmelia conspersa.* A strain of *Proteus vulgaris* was inhibited by *Parmelia physodes* and by *Umbilicaria papulosa. Aerobacter aerogenes* and *Serratia marcescens* were not inhibited by the lichens which were antibiotic for *Alcaligenes* and *Proteus.* The lichens *C. atlantica, C. caroliniana, C. cristatella, C. Grayi,* and *Parmelia physodes,* which are active against *B. subtilis,* were tested also with *B. cereus* and *B. vulgatus,* and were found inhibitory for these species of bacteria. Species which were inactive when tested with *E. coli* and *S. aureus* are the following: *Baeomyces roseus, C. apodocarpa, C. bacillaris, C. clavulifera, C. conista, C. pleurota, C. Robbinsii, C. subcariosa, C. submitis, C. verticillata, Leptogium* sp., *P. rudecta* and *Parmeliopsis aleurites. Baeomyces roseus, C. apodocarpa* and *C. subcariosa* were inactive also with *B. subtilis.* It is possible that activity would be found if these lichens were tested against other bacteria.

The activities of different lichens which were extracted with chloroform, ethyl alcohol or ether are recorded in table 2. The equivalent weights of moist lichen material (200, 300 or 500 mg.) extracted with the solvents and finally taken up in 1.0 or 1.2 ml. of phosphate buffer solution are listed under each species in the table. Although the data are not strictly quantitative for chloroform and alcohol because of some losses incurred during transfer of the solutions, the results indicate clearly that active antibacterial substances can be extracted from lichens with convenient solvents and passed on into an aqueous test medium. That more than one antibiotic compound may exist in lichens is suggested by the fact that both *S. aureus* and *B. subtilis* are inhibited by extracts from *C. Grayi, Parmelia physodes* and other species, while substances obtained from some species of *Cladonia* inhibit *B. subtilis* but not *S. aureus.* Furthermore, extracts from *C. furcata, C. papillaria* and *Umbilicaria papulosa* inhibit *S. aureus* but are inactive against *B. subtilis.* The inhibition of some Gram-negative bacteria by selected species of lichens lends further support to the theory of multiple substances. Until more information becomes available about the nature of these antibacterial compounds, no names will be suggested for them as additions to the already confused terminology of antibiotics.

Characteristic zones of bacterial inhibition obtained with ether extracts, transferred to phosphate buffer for making the tests, are shown in figure 1. It should be mentioned here that with some lichens inhibition was obtained with both ether and aqueous extracts; in other instances antibiosis was found with only the ether or the phosphate buffer solution. As an example, an ether extract of *C. glauca* in figure 1 shows no antag-

TABLE 2

ANTIBIOTIC ACTIVITY OF LICHEN EXTRACTS TESTED AGAINST *B. subtilis*. WEIGHT OF MOIST LICHEN IS GIVEN AS MG. PER ML. OF BUFFER SOLUTION. DIAMETERS OF BACTERIAL INHIBITION ZONES ARE EXPRESSED IN MM.

SPECIES OF LICHEN	PRIMARY SOLVENT	INHIBITION ZONES
C. caroliniana	Chloroform	23
(500 mg./1.2 ml.)	Alcohol	19
	Ether	25
C. cristatella	Chloroform	26
(200 mg./1.2 ml.)	Alcohol	27
	Ether	28
C. incrassata	Chloroform	25
(200 mg./1.2 ml.)	Alcohol	25
	Ether	29
C. Grayi	Chloroform	23
(300 mg./1.2 ml.)	Alcohol	24
	Ether	25
Parmelia physodes	Chloroform	15
(200 mg./1.2 ml.)	Alcohol	15
	Ether	18
C. strepsilis	Ether	20
(200 mg./1.0 ml.)		
C. nemoxyna	Ether	20
(200 mg./1.0 ml.)		
Parmelia conspersa	Ether	23
(200 mg./1.0 ml.)		
Peltigera sp.	Ether	16
(200 mg./1.0 ml.)		
Physcia sp.	Ether	23
(200 mg./1.0 ml.)		
Ramalina sp.	Ether	21
(200 mg./1.0 ml.)		
C. uncialis	Ether	19
(200 mg./1.0 ml.)		

onism for *B. subtilis*, but an aqueous extract made directly from the same lichen gave slight inhibitory action on the same species of bacteria. Hence we have listed *C. glauca* in table 1 as being active against *B. subtilis*.

Without additional information it is impossible to say whether the antibiotic activity of lichens should be attributed to the algal or fungal member of the complex. Almost nothing is known about the anabolism of the

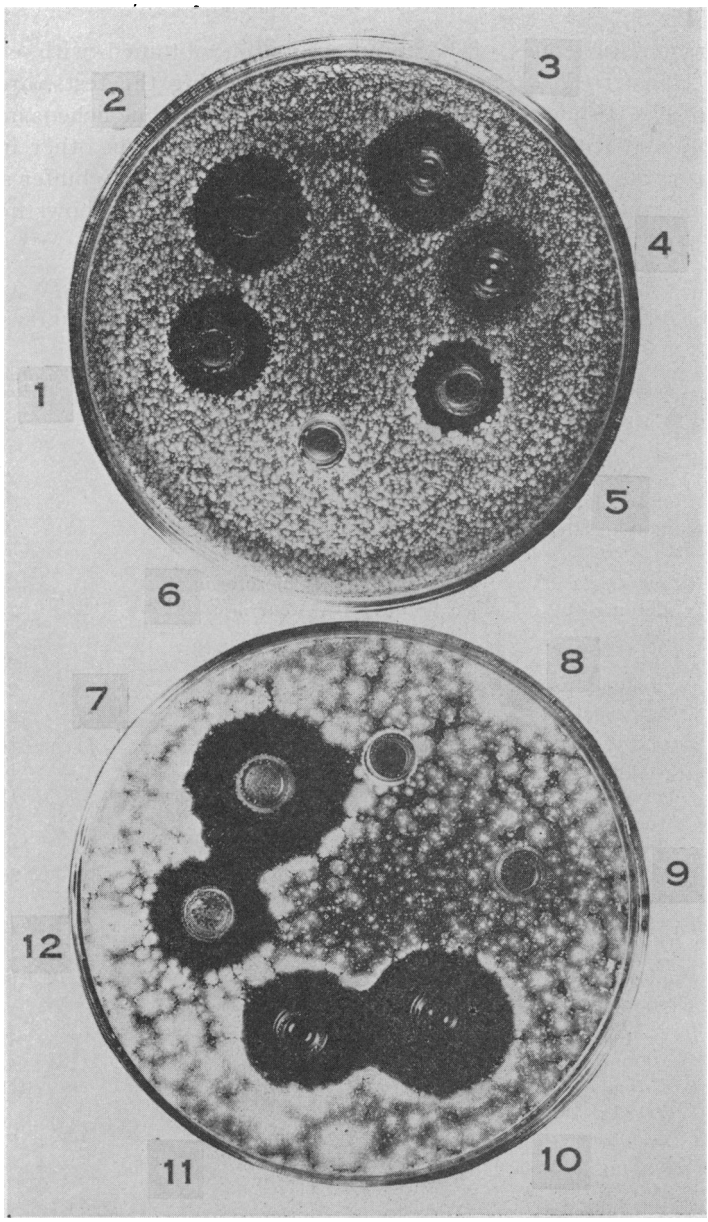

FIGURE 1

Antibacterial action of extracts of lichens. Above, lichen substances extracted with ether were tested in aqueous phosphate buffer against *B. cereus;* below, extracts tested against *B. subtilis.* Sources of the extracts: 1, *Parmelia physodes;* 2, *C. cristatella;* 3, *C. caroliniana;* 4, *C. atlantica;* 5, *C. Grayi;* 6, *C. coniocraea;* 7, *Parmelia consperse;* 8, *C. apodocarpa;* 9, *C. glauca;* 10, *C. caroliniana;* 11, *Physcia* sp.; 12, *Ramalina* sp.

components or the roles of the various substances formed in the lichen body. Chemical antagonism would appear to constitute a possible defense mechanism operating in the struggle for existence among these primitive plants.

One question of considerable importance emerges from the data reported in this paper when considered in the light of earlier work done on the structural chemistry of the lichen acids by Zopf,[6] Asahina,[7] and others. Do the characteristic lichen acids possess antibacterial activity or are the antibiotic properties of lichens related to traces of other unidentified substances synthesized by these plants? Some of the known lichen compounds possess certain structural features in common with antibacterial substances which have been isolated from molds, but whether these are responsible for the antibiotic phenomena which we have observed can be tested only by further experiments which are in progress.

Summary.—Antibacterial activity of extracts from 42 species of lichens was determined with the cup plate procedure. Twenty-seven species were found active against *S. aureus* or *B. subtilis*, 4 species inhibited *P. vulgaris* or *Alcaligenes fecalis*, but none showed antagonism against *E. coli*.

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ACTIVITY AND NUTRITIONAL DEPRIVATION

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Animals in general exhibit increased activity when in want of food. This is in fact the indispensable sign of hunger. It has been observed in widely different organisms from mammals to protozoa;[1] in man and other animals during sleep;[2] in dogs,[3] guinea pigs,[4] and pigeons[5] after decerebration; and in rats from which virtually the entire stomach had been removed.[6] Increased activity therefore holds a primary position in the hunger reaction, persisting in higher animals which lack the equipment for either hunger sensations or the hunger contractions of the stomach.