Mosses as Possible Sources of Antibiotics

Abstract. An examination of 12 species of mosses has indicated that three produce substances capable of inhibiting the growth of various bacteria and other fungi. The method of extraction included several solvents. The extracts were not consistent in their antagonistic activity against the various species of microorganisms, nor were those that displayed antibiotic action always effective against the same organisms. Results indicate unstable products as well as physiological variation in the mosses.

In recent years many possible sources of antibiotics have been explored. These include flowering plants as well as cryptogams such as algae and lichens, in addition to the more highly advertised fungi. Examination of the literature reveals few cases of parasitism of fungi upon bryophytes. Thus it has been felt that assay might reveal some special substance which would prevent the growth of parasites. The suggestion that bryophytes might contain such substances has been made by numerous bryologists.

Earlier investigations have shown that certain products of *Sphagnum portoricense, S. strictum, Conacephalum conicum,* and *Dumortiera hirsuta* have inhibitory powers (*1*), although *Conacephalum conicum* had previously been reported as producing little or no inhibitory substance (*2*).

The mosses that were tested are: *Hygroamblystegium irrigum, Bryum argenteum, Pohlia wahlenbergii, Grimmia wrightii, Anomodon rostratus, Orthotrichum rupestre, Ceratodon stenocarpus, Bryum pallescens, Hedwigia albicans, Sphagnum* sp., *Mnium cuspidatum,* and *Polytrichum* sp. All except the last three were collected locally.

Two hundred grams of whole moss were washed by hand, rinsed in distilled water, and divided into 40-gram aliquots. Each aliquot was then immersed in 70 ml of a different extraction solvent and macerated in a Waring blender. The following extraction solvents were used: distilled water, 95-percent ethanol, acetone, chloroform, and 0.9-percent NaCl solution. After 5 minutes' blending at high speed, each extract was filtered through four layers of cheesecloth, and the filtrate was centifuged. The supernatant and residue were saved for testing. A special method designed from a technique employed by Carlson and Douglas (*3*) was used in preparing the 0.9-percent NaCl extract for testing. The supernatant was divided evenly among three separate test tubes. An equal volume of 1.5-percent H_2SO_4 was added to the first test tube. To the second was added an equal volume of a buffer solution (*p*H 9; 2-percent $Na_2P_2O_7$), and the solution in tube No. 3 had nothing added. The three tubes were placed in the refrigerator for 24 hours. Before testing, the 1.5-percent H_2SO_4 mixture was neutralized with 4-percent NaOH.

The extracts were tested by a modification of the method used by Lucas *et al.* (*4*). The test organisms were grown in tryptose broth (Difco) with the exception of *Phytomonas phaseoli,* which was grown in nutrient broth. The corresponding agar medium was used in the plate examination.

Plates for the examination of the extracts were prepared by flooding the agar surface with 1 ml of an 18 hour broth culture of the test organism and allowing them to stand for 10 minutes. The plates were dried by draining off the excess broth and inverting the plates at a 45° angle with the tops removed. The plates remained in this position for 15 minutes. The discs were prepared by immersing filter paper discs with a 6-mm diameter in the unsterilized extract for 5 seconds. The discs were then dried at room temperature and placed on the inoculated agar surface of the assay plates. These plates were incubated at a suitable temperature for the usual "overnight" period, which did not exceed 18 hours. Zones of inhibition, where found, were recovered as the diameter of the zone and disc. When inhibition occurred with the 4 screening organisms, antibiotic activity of the extract was subsequently tested on six other organisms.

Of the mosses tested, only three showed positive evidence of inhibition. Two of these, *Anomodon rostratus* and *Orthotrichum rupestre,* are shown in Table 1. The supernatant portion of the 95-percent ethanol extract of the third, *Mnium cuspidatum,* produced a 12-mm zone against *Micrococcus flavus* and a 10-mm zone against *Streptococcus pyogenes.* A second test of *Orthotrichum rupestre* collected from the same station but 6 weeks later than the original collection gave results only with the supernatant portion of distilled water in which a 23-mm zone was produced against *Streptococcus pyogenes* and with the supernatant portion of 95-percent ethanol in which a 10-mm zone was produced against *Candida albicans,* a 10-mm zone against *Micrococcus flavus,* and a 16-mm zone against *M. rubens.* Evidence that several compounds, rather than a single one, are involved is shown by the fact that extracts of the same moss species by different solvents gave different results (*5*).

Table 1. Results of screening various moss extracts for antibiotic activity against nine microorganisms. Solvents: A, distilled water; B, 95-percent ethanol; C, acetone; D, chloroform; E, 0.9-percent NaCl solution; E_1, 0.9-percent NaCl extract in 1.5-percent H_2SO_4; E_2, 0.9-percent NaCl extract in *p*H 9 buffers. S, supernatant; R, residue. Numbers denote radial zone diameters in millimeters; P, partial inhibition; x, no test.

	Anomodon rostratus							*Orthotrichum rupestre*						
	A	B	C	D	E	E_1	E_2	A	B	C	D	E	E_1	E_2
	S R	S R	S R	S R	S R	S R	S R	S R	S R	S R	S R	S R	S R	S R
Staphylococcus aureus	0 0	0 0	0 8	9 9	8 0	0 x	0 x	9 P	11 0	9 9	8 0	8 0	0 x	0 x
Salmonella pullorum	P 0	0 11	0 8	8 7	0 0	8 x	0 x	0 0	0 0	0 0	0 0	0 0	0 x	0 x
Phytomonas phaseoli	0 0	0 8	9 8	9 9	8 10	9 x	0 x	0 0	P 0	10 9	8 0	0 0	0 x	0 x
Candida albicans	0 0	0 0	0 0	0 0	0 0	0 x	0 x	8 8	9 0	10 0	0 0	0 8	8 x	8 x
Salmonella paratyphi	0 0	0 0	0 0	0 0	0 0	0 x	0 x	13 0	10 0	10 0	8 9	9 0	9 x	8 x
Micrococcus flavus	0 0	10 0	0 0	0 0	0 0	0 x	0 x	9 0	9 0	10 0	8 0	0 8	0 x	0 x
Shigella flexneri	0 0	P 0	P 0	0 0	0 0	0 x	0 x	9 0	10 0	9 0	0 0	0 0	8 x	8 x
Micrococcus rubens	0 0	0 0	0 0	0 0	0 0	0 x	0 x	0 0	P 0	0 0	0 0	0 0	0 x	0 x
Streptococcus pyogenes	0 0	P 0	P 0	0 0	0 0	0 x	0 x	P 0	P 0	P 0	0 0	0 0	0 x	0 x

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References and Notes

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