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*ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES. X. FOMES JUNIPERINUS SCHRENK**

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In previous publications[1] from this laboratory, three isolations[2] of *Fomes juniperinus* were reported to evidence antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.*

Each of these isolations was grown in Fernbach flasks on a corn steep medium.[3] The culture liquids had antibacterial activity of 32 dilution units per ml. (as measured against *Staph. aureus*) in $2^1/_2$ months after inoculation, and 64 to 256 dilution units after 3 months or more. Supplementing the corn steep medium with 1.0 g. of fish solubles (Van Camp Laboratories) per liter did not improve the development of antibacterial activity. The corn steep medium without the Dox minerals was no more satisfactory than the complete medium. Omitting the sodium nitrate from the complete medium was detrimental, in contrast to the results obtained with the production of pleuromutilin by *P. mutilus.*[4] Shake cultures were unsuccessful because of the slow growth of the fungus.

Through the courtesy of Ross W. Davidson six additional strains[5] of *F. juniperinus* were obtained which were more active producers of antibacterial substances under our conditions. Some of these produce in 2 to $2^1/_2$ months culture liquids with 128 to 256 dilution units as measured against *Staph. aureus.* On reflooding the mats with fresh corn steep medium, culture liquids with an activity of 256 dilution units were obtained in three weeks. No correlation between vigor of growth of the various strains and antibiotic activity was noted.

Isolation of a Crystalline Antibacterial Substance.—A crystalline compound, which accounts for most of the antibacterial activity, was isolated from the culture liquid of eight of the strains of *F. juniperinus.* (Strain 72028-S was not used.) The compound, which has been named *fomecin A*, has the molecular formula $C_8H_8O_5$. At least one other antibacterial substance may be present in the culture liquid.

Antibacterial material was not readily extracted from the culture liquid by organic solvents although at low pH some extraction was accomplished by ethyl acetate. The active material was adsorbed quantitatively on Norit A and about 30 per cent was eluted by aqueous acetone.

The procedure used for the isolation of fomecin A was as follows: Batches of active culture liquid of about 20 liters were adjusted to pH 1 to 2 by the addition of concentrated HCl. The acidified solution was stirred with Norit A, 25 g. per liter, for half an hour and allowed to stand overnight in a cold room at about 7°C. The charcoal was filtered off and eluted three times with 80 per cent acetone using 10 per cent of the volume of the original culture liquid for the first elution and 5 per cent each time for the second and third elutions. The eluates were combined and the acetone removed under reduced pressure; the water bath was kept at from 50° to 60°C. The aqueous solution remaining (about 5 per cent of the volume of the original culture liquid) was subjected to a modified ten funnel counter-current distribution between water and ethyl acetate. The modification consisted in continuing the distribution after all ten funnels had been equilibrated, until all the water phases had passed through the tenth ethyl acetate phase. Most of the pigment remained in the aqueous phase and was carried through in the original water layer. Crude crystalline fomecin A was usually obtained from the ethyl acetate phase of the last six funnels. A yield of 0.36 g. of crude crystalline fomecin A was obtained per liter of culture fluid (Ochoco strain) which had an activity of 256 dilution units per milliliter as measured against *Staph. aureus*. This amount of crystals accounted for less than 10 per cent of the activity of the original culture liquid.

Chemical Properties of Fomecin A.—Crude crystalline fomecin A obtained from the counter-current distribution was crystallized from ethanol-water, ethanol-benzene, acetone-benzene or ethyl acetate. The color and form of the crystals varied strikingly in different recrystallizations. The color ranged from light cream or off-white to a bright orange. Different forms obtained were heavy hexagonal prisms, thin triangular plates, and rods or sharp needles. The various forms and colors seemed to be associated with the type of solvent (more or less polar) and with concentrations at the inception of crystallization. It was frequently possible to induce crystallization of a desired form by seeding with that form. Fomecin A was found to be a weakly acidic compound, sparingly soluble in water (roughly 1 mg. per ml.), slightly more in ethanol, acetone and ethyl acetate, less in chloroform and very slightly in benzene. The compound was stable to short boiling in aqueous solutions at neutral or acid pH. Above pH 8 the solution darkened and with a few seconds of boiling about half of the antibiotic activity was lost. In an open capillary or sealed in vacuum fomecin A did not melt but charred gradually above 160°C. Its solution in 95 per cent ethanol was optically inactive. It showed specific absorption in the ultra-

violet with maxima at 241 mμ (ε = 10,760) and 305 mμ (ε = 14,260). Studies which will be reported separately suggest a quinoid type of structure.

Two separate samples were used for analysis.[6] One (sample 1) was obtained from the culture liquid of *F. juniperinus* (CBS) and was recrystallized from ethyl acetate. The other (sample 2) was obtained from the culture liquid of strain Ochoco and was recrystallized three times from ethanol-water. Both samples were dried *in vacuo* at 78°C. for 3 hours.

	C	H	O	NEUT. EQUIV.	MOL. WT.
Found:					
Sample 1	52.59	4.49	...	173	...
Sample 2	52.28	4.49	43.47	...	189
Calcd. for $C_8H_8O_5$ with one acidic grouping	52.18	4.38	43.44	184	184

A Second Active Principle.—A second antibacterial substance in the culture liquids and in the eluate is suspected on the following basis. The culture liquid and eluate were from 4 to 8 times as effective on *Staph. aureus* as on *Mycobacterium smegma*. Fomecin A was 32 times as active on *Staph. aureus* as on *Myco. smegma*. The activity on *Staph. aureus* of the first water extract passing through the counter-current system was from 2 times to $^1/_8$ that on *Myco. smegma*. It appears probable that a substance with greater relative activity on *Myco. smegma* than fomecin A accumulated in the water phase of the first tubes of the counter-current distribution.

It is possible to follow fomecin A in the counter-current distribution by the ultra-violet absorption peak at about 300 mμ. The first one to four water extracts passing through the counter-current system show a peak at 260–270 mμ and are actively antibacterial. It is not yet clear whether the ultra-violet absorption peak at 260–270 mμ belongs to a hypothetical fomecin B or whether it is associated with a compound which is neither fomecin A nor B.

Antibacterial Activity of Fomecin A.—The antibacterial activity of fomecin A expressed as the minimum concentration in micrograms per milliter that inhibited the growth of bacteria for 24 hours (Mycobacterium 42 hours) was as follows:

ANTIBACTERIAL ACTIVITY

Staphylococcus aureus	8–16
Bacillus mycoides	250
Bacillus subtilis	31–63
Mycobacterium smegma	250–500
Escherichia coli	125
Klebsiella pneumoniae	63
Pseudomonas aeruginosa	125

Antifungal Activity.—Antifungal activity of fomecin A was determined by serial dilution in a peptone medium at pH 6.0 using spore suspensions of fungi as inoculum.[7] Trichophyton was incubated at 30°C., the others at 25°C. The compound had little activity on the fungi tested. The minimum inhibitory concentration of fomecin A was 500 μg. per ml. for *Trichophyton mentagrophytes*. The growth of the following fungi was not inhibited by as much as 500 μg. per ml.: *Aspergillus niger*, *Chaetomium globosum* (USDA 1042.4), *Gliomastix convoluta* (PQMD4c), *Memnoniella echinata* (PQMD1c), *Myrothecium verrucaria* (USDA 1334.2), *Penicillium notatum* (832), *Phycomyces blakesleeanus* (+ strain), *Saccharomyces cerevisiae* (188) and *Stemphylium consortiale* (PQMD 41b).

Antiviral Activity.—Fomecin A was tested by the paper disc method[8] for activity against 59 bacteriophages and one actinophage (*Streptomyces griseus*). Inhibition of phage activity was observed on one of four streptococcal phages, one of six enterococcal phages, three of sixteen phages of *E. coli*, two phages of *Salmonella schottmuelleri*, one page of *Eberthella typhosa*, seven of eleven phages of *Vibrio cholerae* and on the actinophage.

Activity in Presence of Blood.—Incubation at 37°C. for 4 hours with 5% whole human blood in beef extract broth reduced the activity of fomecin A from 64 dilution units to 2 dilution units.

Animal Toxicity.—In preliminary tests using Carworth male white mice weighing on an average 18–20 g., intravenous injection of fomecin A in doses up to 1 mg. per mouse (ca. 50 mg. per kilo) had no apparent ill effect. Five groups of mice, six in a group, were used for the tests. All mice were treated by intravenous injection into a tail vein. One group, used as control, received 0.5 ml. of 0.9 per cent saline. Groups two to five received the same volume of saline containing 1.0, 0.5, 0.25 and 0.1 mg. of fomecin A, respectively (the solutions were adjusted to pH 7.6 with sodium hydroxide). After one week all mice appeared normal.

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[1] Robbins, W. J., Hervey, Annette, Davidson, R. W., Ma, Roberta, and Robbins, W. C., *Bull. Torrey Bot. Club*, **72**, 165–190 (1945). Hervey, Annette, *Ibid.*, **74**, 476–503 (1947).

[2] Two of these, 72028-S and CBS, were obtained from R. W. Davidson. The third, F-3765, was furnished by Dr. Mildred K. Nobles and came originally from Baarn, Holland. It is possible that strains CBS and F-3765 are from the same isolation.

[3] The complete medium contained per liter, 1.5 g. KH_2PO_4, 0.5 g. KCl, 0.5 g. $MgSO_4 \cdot 7H_2O$, 3 g. $NaNO_3$, 40 g. dextrose and 5 g. Staley special nutrient 114 (corn steep).

[4] Kavanagh, F., Hervey, Annette, and Robbins, W. J., these PROCEEDINGS, **37**, 570–574 (1951).

[5] These strains were Whitman A, B and C, from sporophores on Juniperus, Whitman National Forest, Oregon, 1950. Ochoco: From *Juniperus occidentalis*. Ochoco Na-

tional Forest, Oregon, Collected by Childs, 1950. 66286: From sporophore on *Juniperus utahensis*, Mesa Verde National Park, Colorado, 1944. 94141: From *Juniperus occidentalis*, City Park Bend, Oregon, 1941. The Ochoco strain was the best for the production of antibacterial culture liquids.

[6] The analysis of sample 1 was made by J. F. Alicino and of sample 2 by the Huffman Analytical Laboratories. The molecular weight was determined by the ebullioscopic method.

[7] Kavanagh, F., Hervey, Annette, and Robbins, W. J., these PROCEEDINGS, **35**, 343–349 (1949).

[8] Asheshov, I. N., Strelitz, Frieda, and Hall, Elizabeth A., *Brit. J. Exp. Path.*, **30**, 175–185 (1949). The authors are indebted to Dr. Asheshov for the tests on antiphage activity.

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*SEXUALITY IN THE ACRASIALES**

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Although there has been an increasing use of members of the Acrasiales as experimental organisms in morphogenetic and physiological research and a wider use of these primitive slime molds for laboratory study in the past few years, the cytological aspects of the life cycles of these organisms were not known. With this situation in mind and knowing the great need of a critical cytological study of the genus Dictyostelium, the author began the present work in the spring of 1951 with the encouragement of Dr. Ralph Emerson, who supplied a culture of *Dictyostelium discoideum* Raper which he had in turn received from Raper.

Earlier works have revealed little or nothing concerning the nuclear behavior in the Acrasiales. It has been generally assumed that the Acrasiales lack sexuality. Bonner,[1] one of the present day researchers on the group, notes that it is accepted today that the Acrasiales differ from the Myxogastrales in lacking sexuality; he also states that Skupienski[2] is the only investigator who does not agree with this concept and that his view is now generally discredited. Skupienski described the pairing and fusion of gametic myxamoebae and the formation of the aggregation by zygotes in *Dictyostelium mucoroides*. He reported a double division by the cells that went into the formation of the sporangium and postulated that this was comparable to tetrad formation in other cryptogams. He stated, however, that it was not possible to clarify the problem because of inadequate methods of fixing and staining at the time. The lack of conclusive proof prevented acceptance of his observations. K. B. Raper, who initiated the significant modern research on Dictyostelium, does not in his numerous