Phenol in Quackgrass Associated with Dalapon

Abstract. *An unknown compound isolated chromatographically from extracts of rhizome tissues of quackgrass which had previously been treated with dalapon resembled phenol. The compound reduced silver nitrate and had an ultraviolet absorption maximum similar to that of phenol and a positive reaction to the LeRosen test. Melting points and infrared absorption maxima for phenylurethane and α-naphthylurethane derivatives of the unknown matched those for the same phenol derivatives.*

Many phenolics and polyphenolics have been observed as constituents of plant materials (*1*). Though in some plants—for example, tobacco (*2*)—these constituents are normally present in small quantities, it has been generally postulated that stress is a causative factor for the induction of phenols. As early as 1929 it was reported that "black-frost" injury of sultana and currant vines resulted in formation of polyhydroxy benzenes (*3*) and that rust resistance in wheat is due to liberation of phenols in the host cells (*4*). Fusarium resistance in *Zea mays* has been linked to formation of phenols at high temperatures (*5*), and plants acutely infected with little-leaf disease also show phenolics (*6*). Last, stress in the form of ultraviolet irradiation inhibits growth and increases the phenol content of lupine seedlings (*7*).

The following work on the isolation of an unknown compound was part of a study on herbicide translocation in quackgrass rhizomes from plants that had been sprayed in the field with dalapon (*8*). Rhizomes were extracted by a modification of the method of Smith (*9*), and the extract was spotted on paper. The paper was then equilibrated for 12 hours above a solvent system of *n*-butanol saturated with 1.5*N* NH_4OH before the chromatograms were developed for 24 hours by ascending chromatography. After the papers had dried, they were sprayed with a silver nitrate chromogenic agent and allowed to develop for 12 hours in the dark; then they were irradiated with ultraviolet light for approximately 2 hours. The unknown apparently reduced the silver nitrate of the papers treated, since it appeared white against a tan background, while dalapon, in the same procedure, appeared blue. The R_F of this unknown compound was approximately 0.95, much higher than that of dalapon, which was approximately 0.28. In order to isolate enough of the unknown for further study, the base lines (11 cm from the base edge) were streaked with extract until they were saturated, and then they were equilibrated and run as stated above.

The controls were identical extracts made from rhizomes of plants that had not been treated with dalapon. These also showed detectable amounts of phenol, but the concentration was significantly lower than it was in the rhizomes from plants that had been treated.

After evaporation of ethanol extracts of the appropriate sections cut from papers not sprayed with the chromogenic agent, a light amber, syrupy liquid remained. This liquid had a definite phenolic odor and an ultraviolet absorption maximum at 271 mμ. Pure phenol, however, gave a peak at 271 mμ with a shoulder at 277 mμ. *m*-Cresol was then run for its absorption spectrum; it gave a maximum at 273 mμ with a very abrupt shoulder at 279 mμ. For further investigation of the unknown, infrared spectra were run in both carbon tetrachloride and dioxane. Results indicated the presence of an aromatic ring compound.

The unknown, pure phenol, and *m*-cresol gave similar results with the LeRosen test (*10*), which employs a formaldehyde-sulfuric acid reagent. To the *o*-phthalaldehyde test of Feigl (*11*), phenol and the unknown gave a yellow-red color while *m*-cresol gave a yellow color.

A crystalline phenylurethane derivative of the unknown, prepared according to standard procedure (*12*), melted at 126°C, while the same derivatives prepared from phenol and *m*-cresol melted at 126° and 124°C, respectively. Infrared absorption spectra of the phenylurethane of the compound isolated from quackgrass corresponded exactly with the same derivative of pure phenol (Fig. 1).

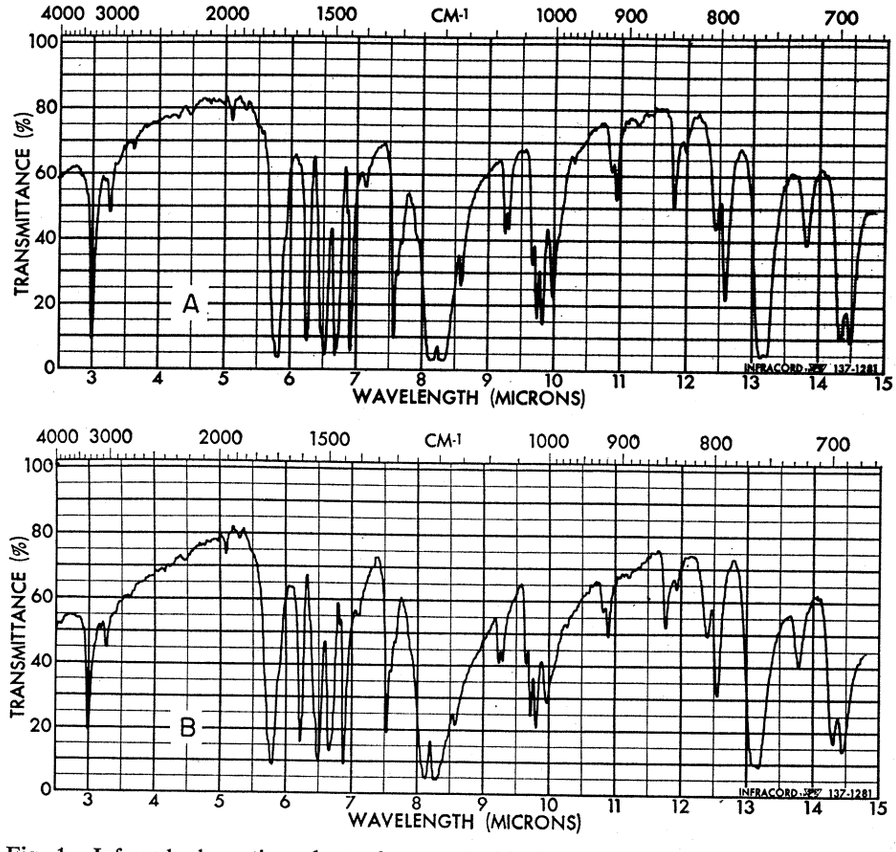

Fig. 1. Infrared absorption charts from a Perkin-Elmer apparatus. *A*, the infrared curve for the phenylurethane derivative of the unknown compound from quackgrass. *B*, the curve for the phenylurethane derivative of pure phenol.

Using Vogel's method (*13*), we prepared α-naphthylurethane derivatives for the compound extracted from quackgrass, for phenol, and for *m*-cresol. The melting points were, respectively, 132°–134°C, 134°C, and 127°C. The literature gives the melting point for phenol as 133°C and for *m*-cresol as 128°C. Infrared absorption spectra for the α-naphthylurethane of the unknown again corresponded with that for pure phenol, while the spectrum for *m*-cresol was decidedly different.

Preliminary tests seemed to indicate a similarity between phenol and the substance isolated from aqueous extracts of quackgrass rhizome tissue by ascending paper chromatography. The agreement between the infrared absorption spectra of prepared derivatives of the unknown material and those of phenol are more striking. Further evidence that the unknown substance is phenol was provided by the carbon, hydrogen, and nitrogen determinations of the phenylurethane derivatives prepared from the rhizome extract and those calculated for the phenol derivative of phenylurethane. For carbon: unknown, 74.37 percent; phenol, 73.22 percent. For hydrogen: unknown, 5.43 percent; phenol, 5.21 percent. For nitrogen: unknown, 6.44 percent; phenol, 6.57 percent. The percentage oxygen was found by the difference and was 13.76, while the calculated percentage was 15.10.

Dalapon has long been recognized as an effective herbicide fairly phytotoxic to quackgrass. Conditions of stress associated with its use are postulated as a causative factor which results in the increase of phenol in rhizome tissue (*14*).

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References and Notes

1. E. C. Bate-Smith, *Sci. Proc. Roy. Dublin Soc.* **27**, 165 (1956); H. Börner, *Naturwissenschaften* **42**, 583 (1955); M. Tomaszewski, *Bull. Acad. Polon. Sci. Ser. Sci. Biol.* **8**, 61 (1960); T. Higuchi, *Physiol. Plantarum* **10**, 356 (1957).
2. R. L. Stedman, *Tobacco* **145**(15), 22 (1957).
3. J. G. Wood, *Australian J. Exptl. Biol. Med. Sci.* **6**, 103 (1929).
4. R. Newton and J. A. Anderson, *Can. J. Res.* **1**, 86 (1929).
5. J. Dufrenoy and T. H. Fremont, *Phytopathol. Z.* **4**, 37 (1931).
6. H. S. Reed, *Am. J. Botany* **25**, 174 (1938).
7. A. S. Garay and F. Soy, *Physiol. Plantarum* **15**, 194 (1962).
8. Kindly supplied by the Dow Chemical Co., Midland, Mich.
9. G. N. Smith, *Down to Earth* **14**(2), 3 (1958).
10. F. Feigl, *Spot Tests in Organic Chemistry* (Elsevier, Amsterdam, ed. 5, 1956), p. 135.
11. ———, *ibid.*, p. 382.
12. R. L. Shriner, R. C. Fuson, D. Y. Curtin, *Systematic Identification of Organic Compounds* (Wiley, New York, ed. 4, 1959), p. 265.
13. A. I. Vogel, *A Textbook of Practical Organic Chemistry* (Longmans, Green, New York, ed. 3, 1957), pp. 683–684.
14. This report is part of the M.S. dissertation of one of us (A.R.L.) at the University of Vermont, Burlington, and was supported in part by funds from the Northeastern Cooperative Regional Research Project (NE-42), Regional Research Fund Hatch Act as amended 11 Aug. 1955. Permission to publish the data has been granted by the Vermont Agric. Expt. Sta. as paper No. 118 in the journal series.
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Breakdown of Rat-Liver Ergosomes in vivo after Actinomycin Inhibition of Messenger RNA Synthesis

Abstract. *Ribosomes isolated from rat liver occur predominantly in the form of aggregates (ergosomes) corresponding to multiples of 73S particles held together by messenger RNA. After injecting rats with actinomycin, these aggregates gradually break down in vivo to 73S monomers and 113S dimers. We conclude that the observed breakdown results from the degradation of messenger RNA and the prevention by actinomycin of the synthesis of new messenger RNA.*

From evidence which was obtained with rat liver ribosomes, we have concluded that the functional unit of protein synthesis is a ribosomal aggregate which we have called ergosome (*1*); it consists of at least five 73*S* particles held together by messenger RNA (mRNA). Exposure of ergosomes to minute concentrations of ribonuclease causes a progressive fragmentation by random cleavage into smaller oligomers $(73S)_4$, $(73S)_3$, and so forth; this process thus results in the accumulation of free 73*S* monomers. This breakdown into smaller aggregates is accompanied by a rapid loss of potentiality for protein synthesis; some residual activity is associated with the 113*S* dimers, whereas the 73*S* monomers are completely inactive. Our conclusion that the ribonuclease-sensitive structure responsible for the integrity of the ergosome is in fact messenger RNA was further supported by experiments, with P^{32}-labeled phosphate, to detect rapid synthesis. An RNA fraction of high specific activity had become associated with purified ribosomes isolated 30 minutes after injecting the radioactive phosphorus. Upon treatment of the ribosomes with ribonuclease, or during incubation to effect the incorporation of amino acids, a large proportion of this rapidly labeled fraction was degraded into acid-soluble products, whereas the bulk of the ribosomal RNA remained stable (*2*). Moreover, dissociation of the labeled ribosomes into subunits at low Mg^{++} concentrations followed by sedimentation analysis in a sucrose gradient gave distribution patterns (*3*) analogous to those obtained with ribosomes from *Escherichia coli* containing labeled mRNA (*4*).

From the rapid synthesis of mRNA in stationary cells it must be inferred that breakdown occurs at the same rate so as to maintain steady-state conditions.

Our experiments were designed to obtain direct evidence for the breakdown in vivo of mRNA associated with ribosomes. Thus, if there is a rapid turnover in vivo of mRNA and if it is also responsible for the structural integrity of ergosomes, one would expect that inhibition of mRNA synthesis should result in the breakdown of ergosomes into 73*S* particles. This prediction was borne out by experiments with actinomycin C_3, a substance which specifically inhibits DNA-dependent RNA synthesis (*5*, *6*).

Actinomycin C_3 the structure of which has been elucidated by Brockmann *et al.* (*7*) is biologically equivalent to actinomycin C_1 (*8*), but differs from C_1 in the peptide chains: C_3 contains D-alloisoleucine in place of the D-valine residue of C_1. The compound named actinomycin D in the older nomenclature is identical with C_1. For injection, a concentrated solution of the crystalline substance (*9*) in ethanol (10 mg/ml) was diluted with saline to a final concentration in the range of 100 to 250 μg/ml.

Male albino rats weighing 350 g were injected intraperitoneally or in the penile vein and kept under observation until they were killed by decapitation 4 to 13 hours later. The livers were removed quickly, weighed, minced with scissors, and homogenized with Littlefield's Medium A (*10*) (2.0 ml/g tissue) in a Potter-Elvehjem device with 12 strokes of a motor driven Teflon pestle. The supernatant fluid (PM) obtained after removing unbroken cells, debris, nuclei, and mitochon-