Alternaric Acid, a Biologically Active Metabolic Product of the Fungus *Alternaria solani*

SEVERAL strains of *Alternaria solani* (E. and M.) Jones and Grout and one of *A. porri* (Ell.) Saw. have been found to produce in liquid media a substance which causes a characteristic 'stunting' effect on developing germ tubes of *Botrytis allii*, in our standard germination test[1], without reducing percentage germination. We have also found that germination of spores of other fungi, notably those of *Myrothecium verrucaria*, is inhibited, so that a more satisfactory assay can be developed. Greatest activity develops in cultures on Czapek–Dox medium containing 10–15 per cent sucrose; sucrose is appreciably superior to glucose or other sugars. From such culture filtrates, using a strain of *Alternaria solani* (No. 408 in our collection), we have isolated a material, for which we propose the name 'alternaric acid', which appears to be responsible for both the antifungal effects mentioned above.

Alternaric acid is obtained by adjusting the culture filtrate to *p*H 3·5 and extracting with chloroform; the gummy residue left after evaporation of the chloroform is dissolved in hot carbon tetrachloride or benzene, and crystalline alternaric acid appears on cooling. Yields of the crude crystalline material of the order of 100 mgm./l. are obtained. Further crystallization from benzene may be necessary to remove a bright orange impurity, and the colourless material can then be purified by repeated crystallization from ethanol or water. It is an optically inactive dibasic acid, m.p. 134° C.; the chemistry of alternaric acid will be reported elsewhere.

Alternaric acid is not appreciably antibacterial. Its antifungal activity is remarkably specific. Germination of spores of *Absidia glauca*, *Myrothecium verrucaria* and *Stachybotrys atra* is inhibited in the range 0·1–1·0 μgm./ml. On the other hand, percentage germination of spores of *Botrytis allii*, *Fusarium cœruleum* and *Penicillium digitatum* is unaffected by concentrations as high as 200 μgm./ml., but a reduction in the rate of hyphal extension, leading to the production of 'stunted' forms, is caused by much lower concentrations. A clearly visible 'stunting' effect on *B. allii* germ tubes is produced by 0·01 μgm./ml. alternaric acid. This type of antifungal activity, in which high specificity is combined with this 'stunting effect', in our experience has only been shown by alternaric acid and by mycophenolic acid. The antifungal specificity of mycophenolic acid has been noted by Gilliver[2].

Alternaria solani is normally parasitic on plants of the family Solanaceæ, but according to Neergaard[3] may also infect plants in other families, such as Cruciferæ and Compositæ. Whipple[4] and Thomas[5] have suggested that *A. solani* produces a diffusible toxin in host (tomato) tissues which causes wilting and collapse of tissues in advance of the actual infection. It is of interest to consider whether alternaric acid may be responsible for this phytotoxic effect. We have introduced alternaric acid into nutrient solutions in which tomato, pea, beet, radish, cabbage, mustard and carrot seedlings were growing; concentrations of 5–10 μgm./ml. caused, after an interval of seven to ten days, a severe wilt followed by death of the radish, cabbage, mustard and carrot seedlings; but the other seedlings were apparently unaffected. Seeds of radish, mustard, tomato, clover and wheat were incubated on agar containing alternaric acid; growth of the radish, mustard and tomato seedlings was severely retarded by 1–5 ugm./ml. alternaric acid; but wheat and clover were somewhat more resistant. Thus, alternaric acid is undoubtedly phytotoxic, but its significance in the etiology of parasitic attack by *A. solani* needs further investigation.

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[2] Gilliver, *Ann. Bot.*, **10**, 271 (1946).
[3] Neergaard, "Danish Species of Alternaria and Stemphylium" (Copenhagen, 1945).
[4] Whipple, Wisconsin Univ. Sums. Doct. Diss., **3**, 65 (1938).
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Synthesis of Cinerone, Cinerolone and of Cinerin-I

As part of a scheme to elucidate by synthetical methods the structures of the cinerins and pyrethrins, the important insecticidal constituents of pyrethrum flowers, one of us in 1946[1] synthesized 2-but-2′-enyl- and 2-but-3′-enyl-3-methyl*cyclo*pent-2-enone (I*a* and I*b*) for comparison with cinerone, which is a degradation product of cinerolone[2]. LaForge and Barthel[3] had previously shown that dihydrocinerone was identical with 3-methyl-2-*n*-butyl*cyclo*pent-2-enone (I*c*), so that only the position and configuration of the side-chain double bond was in question. They had assigned this to the 2′ position on the somewhat uncertain basis of terminal methyl values[2]. This assignment accords with the ultra-violet absorption spectrum of (+)-cinerolone, which excludes the 1′ position; and the non-identity of cinerone with synthetic I*b*, which excludes the 3′ position[1]. We have confirmed the but-2′-enyl side-chain in (+)-cinerolone by ozonization, using a specimen prepared from the semicarbazone (kindly provided by Dr. T. F. West). This yielded acetaldehyde together with a small proportion of formaldehyde. The latter product indicates a small proportion of contaminant, probably pyrethrolone.

The synthetic substance I*a* was not identical with cinerone, although showing fairly close resemblance (see the accompanying table), for both the semicarbazones and *p*-nitrophenylhydrazones gave depressions of melting point. It was concluded that cinerone had the opposite geometrical configuration of the side-chain double bond to the synthetic ketone[1]. The synthesis had started from crotonaldehyde, which Gredy and Piaux[4] have shown by Raman spectroscopy to possess the *trans* configuration. Consideration of the subsequent steps leads