

774. *Alternaric Acid. Part I. Purification and Characterisation.*

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Alternaric acid, $C_{21}H_{30}O_8$, a phytotoxic metabolite of *Alternaria solani*, is an optically inactive unsaturated dibasic acid. Its ultra-violet and infra-red absorption spectra and other evidence show that one of the acidic functions is due to the presence of a β -dicarbonyl grouping.

THE production by certain strains of *Alternaria solani* (Ell. and Mart.) Jones and Grout of a substance with remarkably specific antifungal activity but low antibacterial properties has been described by Brian, Curtis, Hemming, Unwin, and Wright (*Nature*, 1949, **164**, 534). The active compound, alternaric acid, is markedly phytotoxic and it is possible that it is, at least in part, responsible for plant diseases caused by *A. solani* (Brian, Elson, Hemming, and Wright, *Ann. Appl. Biol.*, in the press; cf. Pound and Stahmann, *Phytopathology*, 1951, **41**, 30). Isolation of the acid has been described by Brian, Curtis, Hemming, Jefferys, Unwin, and Wright [*J. Gen. Microbiol.*, 1951, **5**, 619; cf. Darpoux, Faivre-Amiot, and Roux (who used a different method of extraction), *Compt. rend.*, 1950, **230**, 993; *Compt. rend. Acad. Agric.*, 1949, **35**, 266].

Alternaric acid, $C_{21}H_{30}O_8$, m. p. 138° , is a highly crystalline, colourless, optically inactive, unsaturated dibasic acid; it contains no methoxyl groups, and forms a monohydrate, m. p. 135° . It is unchanged in hot water but was immediately hydrolysed by boiling dilute mineral acids or sodium hydroxide. Acid hydrolysis rapidly gave one molecule of carbon dioxide with some indications of the liberation of a second molecule, whereas alkaline hydrolysis gave a number of volatile products including acetone. A complex mixture of acidic products remained in each case, as yet unidentified.

TABLE 1. *Infra-red absorption.*

Compound	Absorption frequency (cm. ⁻¹)		
	C=C	C=O	OH
Alternaric acid	1650	1710, 1732	3150, 3430
Alternaric acid monohydrate	1650	1710, ca. 1727	2490, 2610, 3480
Methyl alternarate	1650	1710, 1738	3500

TABLE 2. *Ultra-violet absorption.*

Solvent	Alternaric acid		Methyl alternarate	
	λ_{max} .	log ϵ	λ_{max} .	log ϵ
0.1N-NaOH	271, 250	4.14, 4.17	273, 250	4.10, 4.17
Water	273, ~253, ~210	4.04, 3.83, 4.06	—	—
Ethanol	274, ~210	4.04, 4.07	273, ~252, ~210	4.05, 3.88, 3.98
Methanol + 5% of HCl	273, ~210	3.99, 3.99	273, ~210	4.06, 4.08

The infra-red spectrum of solid anhydrous alternaric acid (Table 1) showed two bands in the C=O stretching region, and two weak bands due to OH groups in the $3\text{-}\mu$ region. The spectrum of the monohydrate was almost identical between 7 and $14\ \mu$ but showed marked differences in the C=O and OH stretching regions.

The effect of varying the pH of the medium on the ultra-violet absorption of alternaric acid is shown in Table 2. The position of the main absorption band of alternaric acid at $273\ m\mu$ is unaffected, but in alkaline solution a second absorption band appears at $250\ m\mu$. Inflexions in the curves at this point were observed when alternaric acid was examined in aqueous or in ethanolic solutions.

Alternaric acid titrated as a dibasic acid but the potentiometric titration curve showed only one point of inflexion. Heating with excess of 0.1N-sodium hydroxide followed by back titration failed to disclose additional acidity. Alternaric acid contains one carboxyl group as shown by a positive hydroxamic acid test and formation of a methyl ester on esterification with methanol and concentrated sulphuric acid; the second titratable acidic function is attributed to a β -dicarbonyl grouping, the presence of which is indicated by

characteristic colour reactions and by the formation of a chloroform-soluble copper complex. Although the orange colour with ferric chloride and the ready production of one molecule of carbon dioxide on acid hydrolysis suggested the presence of a tetrionic acid grouping in alternaric acid, this is considered to be excluded by the ultra-violet and infra-red spectroscopic evidence (Herbert and Hirst, *Biochem. J.*, 1935, **29**, 1881; Duncanson, personal communication; Grove, unpublished results). Nevertheless, the presence of an enolic system is shown by the ultra-violet absorption spectra, and the peak at 250 μ which appears in aqueous alkali but not in methanolic hydrochloric acid, in which enolisation and ionisation are suppressed, is attributed to the ionised enolic form of the β -dicarbonyl grouping.

The monomethyl ester of alternaric acid titrated as a monobasic acid, pK 4.35 (at 25°). It behaved similarly to alternaric acid in its colour reactions and ultra-violet absorption (Table 2). The infra-red spectrum (Table 1) also was similar to that of alternaric acid but showed only a single strong OH absorption. Both the acid and the methyl ester appear to be in the unenolised form in the solid state.

Zerewitinoff determinations in anisole showed the presence of four active hydrogen atoms in alternaric acid and three in the methyl ester. Thus, of the eight oxygen atoms in alternaric acid, four are contained in the carboxyl and β -dicarbonyl groupings and (probably) two in hydroxyl groups, leaving two, the function of which has not yet been determined.

In a microhydrogenation experiment with a palladium catalyst, alternaric acid absorbed hydrogen equivalent to the saturation of 3.6 double bonds and this figure undoubtedly includes the reduction of one or more carbonyl groups. Nevertheless, the behaviour of the acid towards bromine water and neutral permanganate in acetone, in conjunction with the infra-red and ultra-violet evidence, indicates the presence of (at least) one ethylenic bond; and it follows from the empirical formula and from what is known of the oxygen function that the presence of an aromatic ring is excluded.

EXPERIMENTAL

Microanalyses are by Drs. Strauss and Weiler, Oxford, and by Mr. W. Brown. M. p.s are corrected. The infra-red spectra were obtained with Nujol "mulls" in the apparatus described by Grove and Willis (*J.*, 1951, 877).

Alternaric Acid.—The crude yellow solid (m. p. 122—128°; 4.0 g.), obtained by chloroform-extraction of culture filtrates, previously adjusted to pH 3.5, of *A. solani* (strain 408) grown on sucrose-nitrate media (Brian *et al.*, *loc. cit.*, 1951) was taken up in hot benzene and a little amorphous insoluble material rejected. The solid which separated on cooling was twice recrystallised from dry benzene, giving a colourless product, m. p. 132° (2.7 g.). The bright yellow benzene mother-liquors were bulked and concentrated, to yield slightly less pure material, m. p. 130—132° (0.6 g.). The first fraction was dissolved in saturated sodium hydrogen carbonate solution (50 ml.) and extracted with ether (removing only a trace of gum); *alternaric acid* was then recovered by acidification of the bicarbonate solution with concentrated hydrochloric acid, dried *in vacuo* at room temperature, and crystallised twice from aqueous methanol. It formed colourless, thin, rectangular plates, m. p. 135—136° (2.4 g.), of the monohydrate (see below). For analysis, specimens were crystallised from absolute methanol or ethanol and formed elongated prisms, m. p. 138° [Found, on a sample dried *in vacuo* over phosphoric oxide (a) at 20°: C, 61.7, 61.6, 61.7, 61.6, 61.3; H, 7.5, 7.3, 7.6, 7.5, 7.3; OMe, 0; C-Me, 12.5%; equiv., 208, 199, 205, 205; *M* (Rast), 408. (b) At 80°: C, 61.5; H, 7.4. (c) At 100°: C, 61.3, 61.3; H, 7.3, 7.3. $C_{21}H_{30}O_8$ requires C, 61.4; H, 7.4; 3C-Me, 11.1; 4C-Me, 14.8%; equiv. (dibasic acid), 205; *M*, 410].

Alternaric acid crystallised from benzene in needles, m. p. 135° [Found, after drying *in vacuo* over phosphoric oxide (a) at 20°: C, 61.9, 62.0, 62.7, 62.4; H, 7.5, 7.3, 7.4, 7.3. (b) At 100°: C, 61.4; H, 7.7]. Specimens dried at room temperature gave high values for carbon due presumably to traces of adsorbed benzene; drying at 100° caused discoloration so that the agreement between the observed and the calculated analytical figures is perhaps fortuitous. The infra-red spectrum of alternaric acid crystallised from methanol or from carbon tetrachloride showed bands at 1560, 1338, 1292, 1270, *ca.* 1225, 1215, 1175, 1149, 1117, 1069, 1000, 975, 955, 932, *ca.* 910, 900, 820, 800, and 760 cm^{-1} in addition to those listed in Table 1.

Alternaric acid crystallised from water or aqueous methanol in colourless, thin, rectangular plates of the *monohydrate*, m. p. 135° [Found, dried at 20° *in vacuo* over concentrated sulphuric acid or phosphoric oxide: C, 58.8, 58.9, 59.0; H, 7.5, 7.7, 7.4%; equiv., 215. $C_{21}H_{30}O_8 \cdot H_2O$ requires C, 58.9; H, 7.5%; equiv., 214 (dibasic acid)]. The unit cell was orthorhombic with dimensions $49.96 \times 5.778 \times 7.754$ Å. The density was 1.270 ± 0.003 , whence the molecular weight (4 molecules per unit cell) is 428 ± 1 . $C_{21}H_{32}O_8$ requires *M*, 428. The infra-red spectrum of the monohydrate closely resembled that of the anhydrous compound, but showed a strong band at 1240 cm^{-1} with a weaker absorption at 1214 cm^{-1} in place of the band at 1215 cm^{-1} with inflexion at 1225 cm^{-1} , in addition to the differences in the C=O and OH stretching regions. The molecule of water of crystallisation was removed by phosphoric oxide *in vacuo* at 80° [Found: C, 61.2, 61.3; H, 7.2, 7.3; at 100°: C, 61.7, 61.5; H, 7.0, 7.6%; *M* (Rast), 435]. The infra-red spectrum of the material dehydrated at 80° was identical with that of anhydrous alternaric acid obtained by crystallisation from absolute methanol.

Zerewitinoff determination on anhydrous alternaric acid in anisole at 20° showed 1.01% active H; there was no further reaction at 80° ($C_{21}H_{30}O_8$ requires 4H, 0.98%).

On hydrogenation in acetic acid with a palladium catalyst, 5.100 mg. of alternaric acid took up 1.14 ml. of hydrogen at 27°/759 mm., corresponding to 3.6 double bonds.

Alternaric acid did not sublime at 130°/10⁻⁵ mm. and considerable discolouration and decomposition took place under these conditions. Alternaric acid was optically inactive (cf. Brian *et al.*, *loc. cit.*, 1951) $\{[\alpha]_D^{25} \pm 3^\circ$ (*c*, 1.02 in acetone; *l* = 1 dm.); $[\alpha]_D^{25} \pm 3^\circ$ (*c*, 0.52 in chloroform; *l* = 4 dm.)}. Alternaric acid was only slightly soluble in cold water; it was very soluble in ethanol, methanol, pyridine, and chloroform, moderately soluble in benzene, ether, and carbon tetrachloride, and insoluble in light petroleum. Alternaric acid was recovered unchanged after 30 minutes' heating under reflux in aqueous solution. It dissolved in sodium hydrogen carbonate solution with evolution of carbon dioxide, and in sodium hydroxide. It gave the hydroxamic acid test (Feigl, "Qualitative Analysis by Spot Tests," 3rd Edn., Elsevier Publ. Co., Amsterdam, 1947, p. 357), a wine-red colour in methanol with titanous chloride solution, and a blue colour which faded rapidly when 5% aqueous titanous chloride solution was added to alternaric acid in methanol-pyridine and the solution shaken vigorously in air (Weygand and Csendes, *Ber.*, 1952, **85**, 45). A concentrated solution of alternaric acid in ethanol gave an orange colour with ferric chloride. Alternaric acid rapidly coupled with diazotised 5-chloro-*o*-toluidine in alkaline solution and formed a deep blue, chloroform-soluble copper complex on treatment with a solution of cupric acetate.

Alternaric acid dissolved in cold concentrated sulphuric acid to a colourless solution but charring took place on warming. It did not give a precipitate with 2:4-dinitrophenylhydrazine sulphate in methanol and decomposed when heated under reflux with Girard's reagent P in ethanol containing 10% acetic acid. It neither reduced Fehling's solution nor restored the colour to Schiff's reagent but reduced warm ammoniacal silver nitrate. Bromine water or 1% potassium permanganate solution in acetone was immediately decolourised at room temperature.

Methyl Alternarate.—To alternaric acid (0.40 g.) in methanol (10 ml.) at 0°, ice-cold concentrated sulphuric acid (1.0 ml.) was added with stirring. After 48 hours at 20° the yellow solution was poured into water (50 ml.), and the precipitated *ester* collected, dried, and recrystallised from methanol (0.28 g.) as colourless prisms, m. p. 106–107° (also from benzene) [Found: C, 62.2, 62.2, 62.3; H, 7.4, 7.6, 7.5, 7.6; OMe, 7.8; active H, 0.77, 0.78%; equiv., 438; *M* (Rast), 435. $C_{22}H_{32}O_8$ requires C, 62.25; H, 7.6; OMe, 7.3; 3H, 0.71%; *M*, 424]; elongated plates, m. p. 103°, $[\alpha]_D^{25} \pm 3^\circ$ (*c*, 1.00 in acetone; *l* = 1 dm.), were obtained from aqueous methanol (Found: C, 62.4, 62.2; H, 7.4, 7.5; OMe, 7.1%; equiv., 414). Methyl alternarate was soluble in sodium hydroxide and liberated carbon dioxide from sodium hydrogen carbonate solution. Like the acid, it gave an orange colour with ferric chloride in ethanol, a wine-red colour with 5% titanous chloride in methanol, coupled with diazotised 5-chloro-*o*-toluidine, and formed a chloroform-soluble violet-blue copper complex with cupric acetate solution.

Acid Hydrolysis of Alternaric Acid.—Alternaric acid (98.0 mg.) was heated under reflux with 2*N*-sulphuric acid (10.00 ml.) in nitrogen at 130°, the issuing gases being passed through 0.1*N*-barium hydroxide. 1.16 Mols. of carbon dioxide were evolved in 2 hours, 1.25 mols. in 5 hours. Titration (*N*-sodium hydroxide) of the residue at 80° to a permanent end-point (slow dissolution of much oily material) disclosed that at the end of the reaction 2 potentially acidic groups were present, one possibly in a lactone ring. No volatile acids were detected on acidification and steam-distillation of this neutralised solution.

Action of Alkali on Alternaric Acid.—(a) 0.1*N*-NaOH at 90°. Alternaric acid (83.4 mg.),

titrated with 0.1027N-sodium hydroxide to phenolphthalein, consumed 3.97 ml. (equiv. wt., 205). 4.04 ml. of the alkali were then added and the solution heated at 90° for 1 hour, cooled, and back-titrated with 0.1025N-sulphuric acid to phenolphthalein; 4.05 ml. were required; thus alternaric acid does not contain a readily opened lactone ring. On the addition of concentrated hydrochloric acid to the neutral solution, alternaric acid (hydrate) was precipitated unchanged.

(b) N-NaOH at 100°. Alternaric acid (0.980 g.) and N-sodium hydroxide (20.48 ml.) were heated under reflux for 1 hour. No condensable gaseous products were evolved (solid carbon dioxide trap). A dark neutral oil rapidly separated and, on cooling and back-titration with 2N-sulphuric acid, 6.80 ml. were required, showing that an additional acid group had been formed during the hydrolysis. The solution was made just alkaline with 0.1N-sodium hydroxide and steam-distilled. From the neutral distillate ether removed 0.1 g. of a fragrant neutral oil. The aqueous solution yielded a dinitrophenylhydrazone (m. p. 118°; 0.1 g.; crystallised twice from ethanol), needles or plates, m. p. and mixed m. p. with authentic acetone dinitrophenylhydrazone, 124° [Found: C, 45.8; H, 4.3; N, 23.7%; *M* (Rast), 243. Calc. for $C_9H_{10}O_4N_4$: C, 45.4; H, 4.2; N, 23.5%; *M*, 238].

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