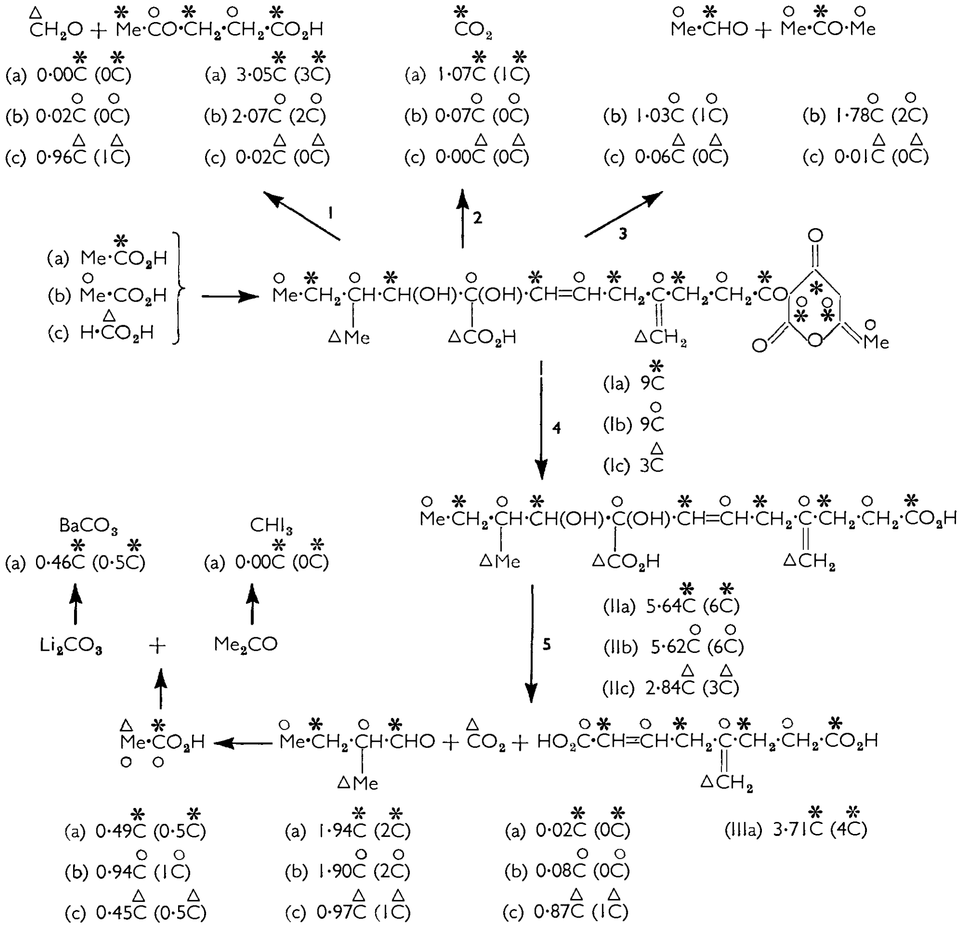
103. *Alternaric Acid. Part V.* Biosynthesis.*

By W. B. TURNER.

Alternaric acid is biosynthesised from nine acetate units and three C_1 units derivable from formate. Incorporation of propionate probably proceeds *via* acetate.

ALTERNARIC ACID[1,2] (I) is a natural product having a branched-chain not obviously derivable from mevalonate. Birch and his collaborators suggested[3] that many such



Reagents: 1, O_3; 2, H^+; 3, OH^-; 4, H_2O_2–OH^-; 5, $Pb(OAc)_4$; 6, Kuhn–Roth oxidation.

---

* Part IV, *J.*, 1960, 3413.

[1] Bartels-Keith and Grove, *Proc. Chem. Soc.*, 1959, 398.

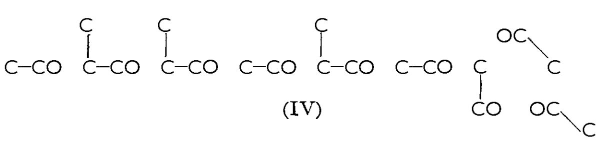
[2] Bartels-Keith, *J.*, 1960, 1662.

[3] Birch, Elliott, and Penfold, *Austral. J. Chem.*, 1954, **7**, 169; Birch, *Fortschr. Chem. org. Naturstoffe*, 1957, **14**, 186.

compounds may be formed by head-to-tail linkage of acetate units followed by the introduction of C_1 units and produced evidence[4] that this is the case for several natural products.

By this mode of biosynthesis, alternaric acid produced in the presence of [^{14}C]-labelled acetate and formate would possess the labelling pattern shown in (I). *Alternaria solani* has been grown on media containing, in separate experiments, $Me{\cdot}^{14}CO_2H$, $^{14}CH_3{\cdot}CO_2H$, and $H{\cdot}^{14}CO_2H$ to give labelled alternaric acid. The incorporation of radioactivity (0·3, 0·3, and 0·2% respectively) was lower than is usual for this type of compound (cf., *e.g.*, ref. 4). The alternaric acid has been degraded by procedures described previously, as shown in the scheme, where the results of radioactivity assays are expressed according to methods used by Birch and his co-workers.[5] The results confirm the expected biogenesis. Agreement between the experimental and the theoretical values is not as close (3%) as might be expected; the deviation is not due to counting errors because (i) repetition of three of the degradations of compound (Ia) gave products whose count-rates differed from those shown in the scheme by only 1—2%, and (ii) there is agreement between the degradations of compounds (Ia and b).

That C_1 units are utilized in the formation of alternaric acid and similar compounds does not exclude the possibility of initial formation of C_3 units (*e.g.*, propionate) which might be incorporated during the chain-building process as shown (IV). It has been pointed out[6] that such a pathway would account for the branched-chain structures of the macrolides, and recent evidence[7] suggests that propionate is directly incorporated into erythromycin. When *A. solani* was grown on a medium containing $Et{\cdot}^{14}CO_2H$ the radioactivity was incorporated into the alternaric acid to a very low degree (0·003%). Acid-hydrolysis of the alternaric acid produced gave the lactone-carbonyl atom as carbon dioxide which was counted as barium carbonate. The ratio of the relative molar activity of the alternaric acid to that of the barium carbonate was 9 : 0·78, providing a strong indication that the radioactivity has been incorporated after preliminary breakdown of the propionate to acetate.


(IV)

When *A. solani* was grown in the presence of [2-^{14}C]-mevalonate the radioactivity was incorporated into the alternaric acid to such a low degree (0·001%) that no degradations were possible. The low incorporation of radioactivity, together with the results of the acetate fermentations, precludes the direct incorporation of mevalonate into alternaric acid.

EXPERIMENTAL

Assays of radioactivity were carried out by essentially standard methods.[5,8] Relative molar activities (r.m.a.) (a), (b), and (c) refer to labelled alternaric acid and its degradation products derived from $Me{\cdot}^{14}CO_2H$, $^{14}CH_3{\cdot}CO_2H$, and $H{\cdot}^{14}CO_2H$ respectively.

[^{14}C]*Alternaric Acid.*—*Alternaria solani* (Ell. and Mart.) Jones and Grout (Strain 1342) was grown on a dextrose–nitrate medium, the labelled substrate (0·5—1 mc; *ca.* 0·125 mc per l. of culture medium) being added in aqueous solution at inoculation. After 15 days, the alternaric acid was isolated by chloroform-extraction of the acidified culture medium and was

---

[4] Birch, English, Massy-Westropp, Slaytor, and Smith, *J.*, 1958, 365; Birch, Fitton, Pride, Ryan, Smith, and Whalley, *ibid.*, p. 4576.

[5] Birch, Massy-Westropp, and Smith, *J.*, 1958, 360 and subsequent papers.

[6] Flynn, Gerzon, Monahan, Quarck, Sigal, Weaver, and Wiley, *Chem. Eng. News*, 1956, **34**, 5138; Woodward, *Angew. Chem.*, 1957, **69**, 50.

[7] Grisebach, Achenbach, and Grisebach, *Naturwiss.*, 1960, **47**, 206.

[8] Popjak, *Biochem. J.*, 1950, **46**, 560.

recrystallised from benzene and then from aqueous methanol to give the monohydrate, m. p. 135—139° [Found: r.m.a. $\times 10^{-3}$, (a) 611·9; (b) 452·0; (c) 306·4].

Degradation of [^{14}C]*Alternaric Acid.*—(i) Hydrolysis with boiling N-sulphuric acid for 2½ hr. gave carbon dioxide isolated as barium carbonate [Found: r.m.a. $\times 10^{-3}$, (a) 72·9 (1C, 68·0); (b) 3·9; (c) 1·6].

(ii) Hydrolysis with N-sodium hydroxide [2] gave acetaldehyde dimethone, m. p. 135—138° [Found: r.m.a. $\times 10^{-3}$, (b) 51·6 (1C, 50·2); (c) 6·0], and acetone 2,4-dinitrophenylhydrazone, m. p. 123—126° [Found: r.m.a. $\times 10^{-3}$, (b) 89·5 (2C, 100·4); (c) 1·5].

(iii) Ozonolysis in methyl acetate at −50° [9] gave formaldehyde dimethone, m. p. 191—192° [Found: r.m.a. $\times 10^{-3}$, (a) 0·29; (b) 2·0; (c) 97·1 (1C, 102·1)], and methyl lævulate 2,4-dinitrophenylhydrazone, m. p. (double) 136—137° and 140·5—141·5° [Found: r.m.a. $\times 10^{-3}$, (a) 207·1 (3C, 204·0); (b) 104·1 (2C, 100·4); (c) 1·9].

(iv) Oxidation with alkaline hydrogen peroxide [9] gave the dibasic acid (II), m. p. 97—99° [Found: r.m.a. $\times 10^{-3}$, (a) 383·7 (6C, 407·9); (b) 282·1 (6C, 301·4); (c) 290·3 (3C, 306·4)].

(v) Oxidation of the dibasic acid (II) with lead tetra-acetate [9] gave (+)-α-methylbutyraldehyde 2,4-dinitrophenylhydrazone, m. p. 135—136° [Found: r.m.a. $\times 10^{-3}$, (a) 132·1 (2C, 136·0); (b) 95·5 (2C, 100·4); (c) 99·2 (1C, 102·3)], barium carbonate [Found: r.m.a. $\times 10^{-3}$, (a) 1·3; (b) 3·9; (c) 89·3 (1C, 102·3)] and 5-methyleneoct-2-enedioic acid (III), m. p. 113—116° [Found: r.m.a. $\times 10^{-3}$, (a) 252·3 (4C, 272·0)].

(vi) Kuhn–Roth oxidation of (+)-α-methylbutyraldehyde 2,4-dinitrophenylhydrazone gave acetic acid, counted as its *p*-bromophenacyl ester, m. p. 85—86° [Found: r.m.a. $\times 10^{-3}$, (a) 33·5 (0·5C, 34·0); (b) 47·4 (1C, 50·2); (c) 45·8 (0·5C, 51·1)]. Pyrolysis of lithium acetate gave barium carbonate (from lithium carbonate) [Found: r.m.a. $\times 10^{-3}$, (a) 31·5 (0·5C, 34·0)] and iodoform [Found: r.m.a. $\times 10^{-3}$, (a) 0·03].

[^{14}C]*Alternaric Acid from* [1-^{14}C]*Propionate.*—*Alternaria solani* was grown as above in the presence of sodium [1-^{14}C]propionate (0·5 mc in 10 l. of culture medium). The resulting alternaric acid monohydrate (Found: r.m.a. $\times 10^{-3}$, 52·9) was hydrolysed as above with N-sulphuric acid to give barium carbonate (Found: r.m.a. $\times 10^{-3}$, 4·59).

I am indebted to Mr. G. L. F. Norris who did the fermentations, and to Mr. B. D. Akehurst for technical assistance.

IMPERIAL CHEMICAL INDUSTRIES LIMITED, AKERS RESEARCH LABORATORIES,
THE FRYTHE, WELWYN, HERTS.
[*Received, August 9th,* 1960.]

---

[9] Bartels-Keith, *J.*, 1960, 860.