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Fungal Detoxication

3. THE METABOLISM OF ω -(2-NAPHTHYLOXY)-*n*-ALKYLCARBOXYLIC ACIDS BY *SCLEROTINIA LAXA**

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The metabolism of ω -(2-naphthyloxy)-*n*-alkylcarboxylic acids by *Aspergillus niger* van Tiegh. has been described in an earlier paper in this series (Byrde, Harris & Woodcock, 1956). By means of a replacement-culture technique it was shown that the acids were hydroxylated in position 6 of the naphthalene nucleus, and that higher members underwent β -oxidation.

The present paper describes an extension of the work to the plant pathogenic fungus *Sclerotinia laxa* Aderh. & Ruhl., the organism causing the brown-rot and blossom-wilt diseases of fruit trees.

EXPERIMENTAL

The materials and reference compounds have already been described (Byrde *et al.* 1956).

Methods

Replacement culture and isolation of metabolites. The method used followed that already described (Byrde *et al.* 1956), with the following modifications. Cultures of *S. laxa*, originally isolated from sour cherry, were grown on a liquid medium composed of: glucose, 40 g.; peptone, 10 g.; KH_2PO_4 , 6.8 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5 g.; CaCl_2 , 0.1 g.; FeCl_3 , 0.02 g.; water to 1 l. The medium was dispensed in 50 ml. lots in 20 oz. medicinal 'flats' and sterilized (10 lb./sq.in. for 20 min.). Each bottle was inoculated with 6 disks (4 mm. in diameter) of mycelium cut from an established culture on solid medium, and incubated at 25° for 7 days. The medium was then replaced by 100 ml. of a solution of the test acid (0.5 mm) in 0.01 M- Na_2HPO_4 . After a further 7 days the substrate was poured off, extracted with ether and examined chromatographically.

Fungitoxicity in vitro. Fungistatic activity was assessed by the rate of mycelial growth of *S. laxa* on an agar medium in which the compounds under test were incorporated. The

liquid medium described above, with the addition of agar (3%, w/v), was dispensed in 50 ml. lots in 100 ml. flasks and autoclaved (10 lb./in.² for 20 min.). The pH was adjusted as necessary, after autoclaving, by the addition to each flask of sterile N-NaOH or N-H₂SO₄ by means of a sterile pipette; the amount required was estimated experimentally from a single flask.

The subsequent procedure followed that described by Byrde & Woodcock (1957).

RESULTS

Identification of metabolites. The metabolism of four unsubstituted and two hydroxylated ω -(2-naphthyloxy)-*n*-alkylcarboxylic acids was studied. Unchanged acid was detected in each instance. Table 1 summarizes the identification of the principal metabolites. In a typical experiment in which δ -(2-naphthyloxy)-*n*-valeric acid (220 mg.) in 0.01 M- Na_2HPO_4 (1800 ml.) was incubated with *S. laxa*, 46 mg. of β -(2-naphthyloxy)propionic acid, together with 45 mg. of unchanged acid, was isolated by large-scale paper chromatography as described previously (Byrde *et al.* 1956). Since β -naphthol was present in trace amounts only, after being washed with aqueous NaHCO_3 the ethereal extracts from a number of experiments were combined to obtain a specimen for mixed m.p. determination.

Fungitoxicity in vitro. The growth of *S. laxa* in glucose-peptone-phosphate agar in the presence of ethanol (1%, v/v) and a series of ω -(2-naphthyloxy)-*n*-alkylcarboxylic acids (0.25 mm) is shown in Table 2.

Since the pK_a value of 2-naphthyloxyacetic acid is 3.7 (Byrde *et al.* 1956), the more reliable comparison of fungistatic activity is that at pH 2.7, where the acids are predominantly undissociated (Simon & Blackman, 1949). The higher pH level

* Part 2: Byrde & Woodcock (1957).

Table 1. *Identification of principal metabolites of ω -(2-naphthylloxy)-*n*-alkylcarboxylic acids incubated with Sclerotinia laxa*

The R_F values were obtained with butanol-ethanol-aq. 3N-ammonia (4:1:5, by vol.)

Substrate	Metabolites	M.p.	R_F	Identification
2-Naphthylloxyacetic acid	None*	—	—	—
β -(2-Naphthylloxy)propionic acid	β -Naphthol	119–120°	0.91	Mixed m.p.
	Unknown compound (I)	—	0.48	—
γ -(2-Naphthylloxy)- <i>n</i> -butyric acid	2-Naphthylloxyacetic acid	153–154°	0.64	Mixed m.p.
δ -(2-Naphthylloxy)- <i>n</i> -valeric acid	β -(2-Naphthylloxy)propionic acid	141–142°	0.67	Mixed m.p.
	β -Naphthol	—	0.92	R_F
	Unknown compound (I)	—	0.48	—
β -(6-Hydroxy-2-naphthylloxy)-propionic acid	Two unknown acidic compounds (II, III)	—	{ 0.25 (II) 0.35 (III)	— —
γ -(6-Hydroxy-2-naphthylloxy)- <i>n</i> -butyric acid	6-Hydroxy-2-naphthylloxy-acetic acid	—	0.37	R_F

* Trace of 6-hydroxy-2-naphthylloxyacetic acid detected chromatographically in two out of five experiments.

Table 2. *Fungistatic activity towards S. laxa of acids of the general formula $C_{10}H_7 \cdot O \cdot [CH_2]_n \cdot CO_2H$ (0.25 mm) in glucose-peptone-phosphate medium*

<i>n</i>	Mean growth rate (mm./day)	
	pH 2.7	pH 7.0
1	0.03	17.69
2	0.00	6.19
3	0.00	5.25
4	1.75	7.07
5	1.60	6.50
6	4.60	15.07
7	4.20	11.13
Control	7.72	19.75
Least significant difference ($P=0.05$)	1.51	5.16

approaches that of the detoxication experiments. The effect of pH on the activity of 2-naphthylloxy-acetic acid is particularly marked.

In subsequent experiments the fungistatic activity towards *S. laxa* of β -(2-naphthylloxy)-propionic acid and its detoxication product, β -naphthol, were compared at two pH levels. The growth-rate data are summarized in Table 3, and the dose-response data, obtained by the probit method for quantitative analysis (Finney, 1952), in Table 4.

DISCUSSION

Although the metabolism of the ω -(2-naphthylloxy)-*n*-alkylcarboxylic acids by *A. niger* involved nuclear hydroxylation, followed by β -oxidation of the side chain, the former process was rarely encountered in the present work with *S. laxa*. Moreover, the presence of substantial quantities of unchanged acids after 7 days' incubation with the fungus indicates a comparatively low rate of β -oxidation. Despite this poor breakdown, β -naphthol was isolated in small amount from β -(2-

naphthylloxy)propionic acid and also detected chromatographically from δ -(2-naphthylloxy)-*n*-valeric acid.

These results are analogous to those of Fawcett, Ingram & Wain (1954), who obtained phenol from flax plants which had been treated with the corresponding phenoxy-*n*-alkylcarboxylic acids, but offer a marked contrast with previous work in which the propionic acid side chain resisted β -oxidation by *A. niger* (Byrde *et al.* 1956; Byrde & Woodcock, 1957). It is significant that when β -(6-

Table 3. *Mean growth rate of S. laxa mycelium in the presence of β -(2-naphthylloxy)propionic acid and β -naphthol at two pH levels*

Conditions were as described in the text			
pH	Compound added	$10^6 \times$ concn. (M)	Mean growth rate (mm./day)
2.7	β -(2-Naphthylloxy)-propionic acid	20	0.13
		10	1.69
		5	7.69
		2.5	9.50
	β -Naphthol	40	0.13
		20	4.56
		10	5.88
		5	10.00
		2.5	11.63
	None	—	16.50
7.0	β -(2-Naphthylloxy)-propionic acid	160	1.13
		80	3.19
		40	7.38
		20	16.25
		10	17.38
		5	19.06
	β -Naphthol	40	0.69
		28	2.13
		20	7.25
		14	9.88
		5	17.50
	None	—	17.81

Table 4. *Dose-response data derived from Table 3*

pH	Compound	Equation of probit regression line	10 ⁵ ED ₅₀ * (M)	Slope of probit regression line	Relative potency*
2.7	β -(2-Naphthylloxy)propionic acid	$Y = 6.11 - 1.89x$	3.88 (1.82-6.64)	-1.89 ± 0.33	100.0
	β -Naphthol	$Y = 6.65 - 1.89x$	7.48 (3.68-11.99)	-1.89 ± 0.33	51.6 (27.3-100.6)
7.0	β -(2-Naphthylloxy)propionic acid	$Y = 10.54 - 3.45x$	43.4 (33.7-53.8)	-3.45 ± 0.52	†
	β -Naphthol	$Y = 10.14 - 4.23x$	16.4 (14.0-21.2)	-4.23 ± 0.85	†

* 5% Fiducial limits are quoted in parentheses.

† No estimate possible, owing to non-parallelism of lines. Ratio of ED₅₀ values, 2.65:1.

hydroxy-2-naphthylloxy)propionic acid was incubated with *S. laxa* no 2:6-dihydroxynaphthalene was detected, which suggests that the retention of an intact propionic acid side chain may be associated with the presence of a hydroxyl group in position 6. The presence of the acidic compounds (II) and (III) referred to in Table 1 suggests that fission of the hydroxyl-containing ring has taken place to some extent. A similar breakdown of 2-methoxynaphthalene by *A. niger* and *S. laxa* has recently been encountered (R. J. W. Byrde, D. F. Downing & D. Woodcock, unpublished work).

The activity of the ω -(2-naphthylloxy)-*n*-alkyl-carboxylic acids C₁₀H₇·O·[CH₂]_{*n*}·CO₂H against *S. laxa* followed a similar pattern to that shown against *A. niger* with a peak in activity at *n* = 2-3. It follows that the conversion of γ -(2-naphthylloxy)-*n*-butyric acid into the corresponding acetic acid is accompanied by a decrease in activity against *S. laxa*. In comparisons of the activities of β -(2-naphthylloxy)propionic acid and β -naphthol, the former was the more active at pH 2.7 where both compounds are present as undissociated molecules. At pH 7.0, however, the acid, which is largely present as the anion, was less active than the naphthol, and hence the conversion of β -(2-naphthylloxy)propionic acid into β -naphthol at pH 8.1 is likely to be accompanied by an increase in activity. Because of the relatively small amounts

of acid which are metabolized, this is unlikely to play an important part in its fungistatic activity towards *S. laxa*.

SUMMARY

1. By the use of *Sclerotinia laxa* in a replacement-culture technique, γ -(2-naphthylloxy)-*n*-butyric and δ -(2-naphthylloxy)-*n*-valeric acids underwent β -oxidation to the corresponding acetic and propionic acids respectively.

2. Similarly, β -naphthol was isolated in small amounts from β -(2-naphthylloxy)propionic acid; the fungistatic activity of the former was higher at pH 7 but lower at pH 2.7.

3. Nuclear hydroxylation by *S. laxa* was virtually absent.

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