152. *Fungal Detoxication. Part VI.* Synthesis of 1-Hydroxy-2-naphthyloxyacetic Acid.*

By E. D. Evens and D. Woodcock.

The synthesis of 1-hydroxy-2-naphthyloxyacetic acid is described. Its formation from 2-naphthyloxyacetic acid in an ascorbic acid–Fe^{2+} system, but not on incubation with mycelia of *A. niger*, has been confirmed.

Hydroxylation in the metabolism of phenoxyacetic acids by *Aspergillus niger* has been shown, by using a replacement culture technique, to take place in more than one position.[1] In contrast, 2-naphthyloxyacetic acid appears to be solely converted into 6-hydroxy-2-naphthyloxyacetic acid,[2] though on hydroxylation by means of a ferrous–ascorbic acid system [3] chromatographic evidence indicated a second hydroxy-compound present to a small extent. The present paper describes a synthesis, after several attempts, of 1-hydroxy-2-naphthyloxyacetic acid, which might have been expected to be a major metabolite. It was also required for test in a plant growth-regulator programme.

The obvious route, catalytic reduction of 1-nitro-2-naphthyloxyacetic acid, is useless because of immediate cyclisation of 1-amino-2-naphthyloxyacetic acid to 2,3-dihydro-2-oxo-1*H*-naphtho [2,1-*b*]-[1,4]-oxazine. Similar treatment of the methyl, ethyl, and phenyl esters also results in ring formation, but the t-butyl ester yields t-butyl 1-amino-2-naphthyloxyacetate in contrast to n-butyl 2,4-dichloro-6-nitrophenoxyacetate which is known to give a lactam.[4] Whilst t-butyl 1-amino-2-naphthyloxyacetate appeared to be diazotised normally, conversion into the hydroxy-acid was not achieved owing to excessive tar formation during decomposition of the diazonium solution. Similar difficulties were met in trying to decompose the diazonium solutions from the methyl and the benzyl ethers of 2-amino-1-naphthol, 1-amino-2-naphthyloxyacetonitrile, and 2-(1-amino-2-naphthyloxy)ethanol.

1-Chloro-2-naphthol-4-sulphonic acid, prepared from 1-amino-2-naphthol-4-sulphonic acid through the 1-diazo-2-oxide, readily gave methyl 2-carboxymethoxy-4-chloro-naphthalene-1-sulphonic acid but attempts to replace the halogen by a hydroxyl or alkoxyl group were unsuccessful. 1-Bromo-4-nitro-2-naphthyloxyacetic acid, however, could be converted into the corresponding 1-hydroxy-acid by heating it with aqueous-ethanolic sodium carbonate at 150°. This acid could be reduced catalytically, but successful diazotisation and deamination of the product was not achieved as planned.

Whilst treatment of 1-acetyl-2-naphthyloxyacetic acid with peracetic acid yielded only 2-carboxycinnamic acid, and the corresponding benzoyl compound was unattacked, this reagent converted 2-acetyl-1-methoxynaphthalene into 2-acetoxy-1-methoxynaphthalene, which on hydrolysis followed by condensation with ethyl bromoacetate, gave 1-methoxy-2-naphthyloxyacetic acid. Demethylation of this acid by refluxing hydrobromic acid was accompanied by cyclisation to 2-oxonaphtho[1,2-*b*]-[1,4]-dioxan, but this gave the required 1-hydroxy-2-naphthyloxyacetic acid on treatment with warm dilute sodium hydroxide solution, followed by careful acidification.

1-Hydroxy-2-naphthyloxyacetic acid, which gave a bright orange dye when coupled with *p*-nitrobenzenediazonium fluoroborate, was shown to be produced from 2-naphthyloxyacetic acid by the ferrous–ascorbic acid system, but was not present in metabolic liquors from cultures of *A. niger* which had been incubated with that acid.

* Part V, Faulkner and Woodcock, *J.*, 1961, 5397.

[1] Byrde and Woodcock, *Biochem. J.*, 1957, **65**, 682; Faulkner and Woodcock, *J.*, 1961, 5397.
[2] Byrde, Harris, and Woodcock, *Biochem. J.*, 1956, **64**, 154.
[3] Udenfriend, Clark, Axelrod, and Brodie, *J. Biol. Chem.*, 1954, **208**, 731.
[4] Cavill and Ford, *J.*, 1954, 565.

Experimental

1-Nitro-2-naphthyloxyacetic Acid.—This was prepared according to Lees and Shedden's directions,[5] the 6-nitro-isomer also produced being separated by crystallisation from acetone–benzene. The pure acid had m. p. 190—191°. The acid chloride, prepared by using thionyl chloride, crystallised from benzene in needles, m. p. 106—107° (Found: C, 54·6; H, 3·0; N, 5·4. Calc. for $C_{12}H_8ClNO_4$: C, 54·3; H, 3·0; N, 5·3%) (Spitzer [6] gives m. p. 94°). The *methyl ester* crystallised from methyl alcohol and had m. p. 105—105° (Found: C, 59·7; H, 4·3; N, 5·4. $C_{13}H_{11}NO_5$ requires C, 59·8; H, 4·2; N, 5·4%).

t-Butyl 1-Nitro-2-naphthyloxyacetate.—A solution of the above acid chloride (13·8 g.) in benzene (10 ml.) and t-butyl alcohol (dried over calcium; 20 ml.) was refluxed with benzene (5 ml.) and anhydrous pyridine (10 ml.) for 5—6 hr. The solution was cooled, an excess of dilute hydrochloric acid added, and the product extracted with ether. The ethereal extract, after being washed with dilute sodium hydroxide and water, was dried (Na_2SO_4) and the solvent removed. The *ester* (12·3 g.) crystallised from benzene–light petroleum (b. p. 40—60°) in yellow plates, m. p. 110—111° (Found: C, 63·3; H, 5·8; N, 4·6. $C_{16}H_{17}NO_5$ requires C, 63·4; H, 5·65; N, 4·6%). A dimorphic *form* occasionally met in recrystallisations had m. p. 147—148° (Found: C, 63·6; H, 5·6; N, 4·7%).

t-Butyl 1-Amino-2-naphthyloxyacetate.—A solution of the above t-butyl ester (4 g.) in tetrahydrofuran (20 ml.) was shaken with Raney nickel in hydrogen until there was no further uptake of gas. The catalyst was separated by centrifugation, and the solvent evaporated under reduced pressure. The oily product solidified (3·5 g.) and crystallised from benzene–light petroleum (b. p. 40—60°) in greenish-yellow prisms, m. p. 71—72° (Found: C, 70·4; H, 7·1; N, 5·1. $C_{16}H_{19}NO_3$ requires C, 70·3; H, 7·0; N, 5·1%). The *3,5-dinitrobenzoate* crystallised from benzene in silky yellow needles, m. p. 203—204° (decomp.) (Found: C, 59·5; H, 4·6; N, 9·3. $C_{23}H_{21}N_3O_8$ requires C, 59·1; H, 4·5; N, 9·0%).

Diazotisation of this amino-ester in dilute sulphuric acid by sodium nitrite or in ethyl alcohol by pentyl nitrite appeared normal, the solution giving a crimson dye when coupled with alkaline β-naphthol. However, pouring the diazonium solution into boiling sulphuric acid of strengths up to 60%, in the presence or absence of copper sulphate, gave only tars.

1-Benzyloxy-2-nitronaphthalene.—A solution of 2-nitro-1-naphthol (4·9 g.) in dimethylformamide (85 ml.) was stirred and refluxed with benzyl chloride (3·5 ml.) and anhydrous potassium carbonate (3·7 g.) for 6 hr., then cooled and poured into water, and the product was extracted with ether. After being washed with dilute sodium hydroxide solution and then water, the extract was dried (Na_2SO_4) and evaporated. The *ether* (6·1 g.) crystallised from methyl alcohol in yellow needles, m. p. 59·5—60° (Found: C, 72·8; H, 4·8; N, 4·8. $C_{17}H_{13}NO_3$ requires C, 73·1; H, 4·7; N, 5·0%).

1-Nitro-2-naphthyloxyacetonitrile.—A solution of 2-naphthyloxyacetonitrile (2 g.) in acetic acid (10 ml.) was stirred at 20—25° during dropwise addition of a mixture of nitric acid (*d* 1·5; 2 ml.) and acetic acid (2 ml.). After 2 hr. the crystalline *nitrile* was collected and recrystallised from acetic acid; it (0·3 g.) had m. p. 174—175° (Found: C, 63·2; H, 3·4; N, 12·1. $C_{12}H_8N_2O_3$ requires C, 63·15; H, 3·5; N, 12·3%).

2-(1-Nitro-2-naphthyloxy)ethanol.—2-Nitro-1-naphthol (10 g.), anhydrous potassium carbonate (7·5 g.), and ethylene chlorohydrin (25 ml.) were heated under reflux for 6 hr. The mixture was cooled, poured into water and extracted with ether. The extract was washed with dilute sodium hydroxide solution and then water, dried (Na_2SO_4), and evaporated. The residual *alcohol* crystallised from aqueous ethyl alcohol in prisms, m. p. 96—97° (Found: C, 61·3; H, 4·6; N, 6·0. $C_{12}H_{11}NO_4$ requires C, 61·8; H, 4·7; N, 6·0%).

2-(1-Amino-2-naphthyloxy)ethanol.—The above nitro-compound (1 g.) in tetrahydrofuran (10 ml.) was shaken with Raney nickel in hydrogen until uptake ceased. The catalyst was separated by centrifugation and the solvent evaporated under reduced pressure. The *amino-alcohol* (0·7 g.) crystallised from aqueous methyl alcohol in plates, m. p. 97—98° (Found: C, 70·5; H, 6·5. $C_{12}H_{13}NO_2$ requires C, 70·9; H, 6·4%).

1-Chloro-2-naphthol-4-sulphonic Acid.—The diazo-oxide (8 g.) prepared from 1-amino-2-naphthol-4-sulphonic acid was boiled with cuprous chloride (8 g.) in concentrated hydrochloric

[5] Lees and Shedden, *J.*, 1903, 758.
[6] Spitzer, *Ber.*, 1901, **34**, 3193.

acid (80 ml.) until nitrogen evolution ceased (about 1 hr.). After dilution with water (700 ml.) the solution was saturated with hydrogen sulphide and left overnight. The filtered solution was then evaporated to dryness, leaving the grey *chloro-acid* (7·9 g.) (Found: equiv., 146·2. $C_{10}H_7ClO_4S,2H_2O$ requires equiv., 147).

4-Chloro-3-ethoxycarbonylmethoxynaphthalene-1-sulphonic Acid.—The above sulphonic acid (6·5 g.) was refluxed for 20 hr. with a solution from sodium (1·6 g.) in ethyl alcohol (50 ml.) and ethyl bromoacetate (3·9 ml.). After evaporation to dryness, the residue was extracted several times with dry acetone, and the combined extracts were evaporated *in vacuo*. Crystallised from n-propyl alcohol (charcoal), the residual ethyl ester had m. p. 245° (decomp.). The *benzylisothiouronium salt* crystallised from 50% aqueous ethyl alcohol in needles, m. p. 153—155° (Found: Cl, 6·7; N, 5·2; S, 12·2. $C_{22}H_{23}ClN_2O_6S_2$ requires Cl, 6·95; N, 5·5; S, 12·5%).

3-Carboxymethoxy-4-chloronaphthalene-1-sulphonic Acid.—Obtained by hydrolysis of the above ethyl ester, this acid crystallised from hot water in needles, m. p. >300°. The *sodium salt* crystallised from water (Found: C, 41·0; H, 3·6; Na, 6·3. $C_{12}H_7NaO_7S,2H_2O$ requires C, 40·7; H, 3·1; Na, 6·5%). The *bisbenzylisothiouronium salt* crystallised from 50% aqueous ethyl alcohol in pale yellow plates, m. p. 173—174° (Found: Cl, 5·3; N, 8·2; S, 14·1. $C_{28}H_{29}ClN_4O_6S_3$ requires Cl, 5·5; N, 8·6; S, 14·8%).

1-Bromo-4-nitro-2-naphthol.—Gradual addition of a mixture of acetic acid (60 ml.) and sulphuric acid (12 ml.) to a cooled suspension of 4-nitronaphthalene-1-diazo-2-oxide [7] (12·3 g.) immediately dissolved the latter. The mixture was poured into a stirred solution of cuprous bromide (8·4 g.) in hydrobromic acid (*d* 1·48; 120 ml.) at 0°, and after 2 hr. it was heated to 75° during 1 hr. and maintained at that temperature until nitrogen evolution had ceased. The mixture was then poured into water (1·5 l.), the precipitate filtered off, washed with water, and dissolved in 5% sodium hydroxide solution, and the solution filtered to remove tar and acidified. The *naphthol* was filtered off, washed with water, dried, and crystallised from benzene (charcoal). It crystallised from light petroleum (b. p. 60—80°) in pale yellow needles (6·8 g.), m. p. 153—154° (Found: C, 44·4; H, 2·1; N, 5·2. $C_{10}H_6BrNO_3$ requires C, 44·8; H, 2·2; N, 5·2%).

1-Bromo-2-ethoxycarbonylmethoxy-4-nitronaphthalene.—1-Bromo-4-nitro-2-naphthol (48 g.) was refluxed for 4 hr. with ethyl bromoacetate (30 ml.) in a solution from sodium (4·1 g.) in ethyl alcohol (480 ml.). The *ester* which separated on cooling was washed with aqueous methyl alcohol and then water and dried. It formed pale yellow needles, m. p. 88—89° (Found: C, 47·8; H, 3·3; N, 3·9. $C_{14}H_{12}BrNO_5$ requires C, 47·5; H, 3·4; N, 4·0%). The *acid*, obtained by hydrolysis, crystallised from methyl alcohol (charcoal) in yellow needles, m. p. 209° (Found: C, 44·1; H, 2·5; N, 4·1. $C_{12}H_8BrNO_5$ requires C, 44·2; H, 2·45; N, 4·3%).

2-Carboxymethoxy-4-nitro-1-naphthol.—A solution of the above bromo-ester (4 g.) in 50% ethyl alcohol (40 ml.) was heated with sodium carbonate (2·4 g.) in a sealed tube at 150° for 7 hr. When cold, the products were acidified with hydrochloric acid and the *acid* was collected, washed with water, and dried at room temperature. It was crystallised by dissolution in a little methyl alcohol and cautious addition of water, forming brownish-yellow needles, m. p. 172—172·5° (decomp.) (Found: C, 54·6; H, 3·2; N, 5·1. $C_{12}H_9NO_6$ requires C, 54·75; H, 3·45; N, 5·3%). The properties of this acid, some samples of which gradually decomposed during normal storage, are being investigated.

2-Acetoxy-1-methoxynaphthalene.—A solution of 2-acetyl-1-methoxynaphthalene (0·4 g.) and toluene-*p*-sulphonic acid (0·05 g.) in acetic acid (4 ml.) was stirred at 35° during dropwise addition of a 6—9% solution (11 ml.) of peracetic acid [8] and left at room temperature for 7 days. It was then diluted with water and extracted with ether, and the extract was washed with sodium hydrogen carbonate solution, dried, and evaporated. The oily residual *ester* crystallised from light petroleum (b. p. 40—60°) in rhombic plates, m. p. 90—91° (Found: C, 72·1; H, 5·5. $C_{13}H_{12}O_3$ requires C, 72·2; H, 5·6%).

1-Methoxy-2-naphthol.—The above acetyl derivative (0·3 g.) was refluxed with methyl alcohol (4 ml.) and 5% sodium hydroxide solution (4 ml.) for 1 hr. The solution was cooled and acidified and the naphthol extracted with ether. It crystallised from light petroleum (b. p. 40—60°) (charcoal) in nacreous plates, m. p. 89—90° (Found: C, 75·4; H, 5·7. Calc. for $C_{11}H_{10}O_2$: C, 75·8; H, 5·7%). Bamberger and Wildi [9] give m. p. 90·5—91°.

[7] Morgan and Evens, *J.*, 1919, 1126.
[8] "Organic Reactions," Wiley, New York, Vol. VII, p. 395.
[9] Bamberger and Wildi, *J. prakt. Chem.*, 1923, **105**, 278.

1-*Methoxy*-2-*naphthyloxyacetic Acid*.—1-Methoxy-2-naphthol (0·4 g.), chloroacetic acid (1 g.), and sodium hydroxide (0·6 g.) in water (5 ml.) were refluxed for 9 hr. and then cooled and acidified and the product was isolated with ether. The *acid* crystallised from aqueous methyl alcohol in prisms, m. p. 118—119° (Found: C, 67·4; H, 5·3. $C_{13}H_{12}O_4$ requires C, 67·2; H, 5·2%).

1-*Hydroxy*-2-*naphthyloxyacetic Acid*.—The preceding methoxy-acid (0·2 g.) was refluxed with hydrobromic acid (3 ml.; *d* 1·49) for 1 hr. The solution was diluted with water and shaken with ether, and the acidic product (0·07 g.) was isolated by washing the ethereal extract (A) with sodium hydrogen carbonate, acidifying the alkaline layer, and re-extracting it with ether. This *acid* crystallised from benzene–light petroleum (b. p. 60—80°) in prisms, m. p. 125—126° (Found: C, 66·5; H, 4·5. $C_{12}H_{10}O_4$ requires C, 66·0; H, 4·6%). Evaporation of the ether (A) above gave 2-*oxonaphtho*[1,2-b]-[1,4]-*dioxan*, which crystallised from benzene–light petroleum (b. p. 60—80°) in prisms, m. p. 90—91° (Found: C, 72·2; H, 4·0. $C_{12}H_8O_3$ requires C, 72·0; H, 4·0%). A solution of this lactone in warm dilute aqueous sodium hydroxide solution, on cooling and cautious acidification, gave an acid, m. p. 120—121°, undepressed on admixture with the above demethylation product.

Chromatography of 1-*Hydroxy*-2-*naphthyloxyacetic Acid*.—Production of this acid from 2-naphthyloxyacetic acid by the Fe^{2+}–ascorbic acid system, and its absence from culture filtrates of *A. niger* which had been incubated with 2-naphthyloxyacetic acid, were demonstrated chromatographically. Location of naphthol spots was achieved by spraying with *p*-nitrobenzenediazonium fluoroborate (1% solution in 20% w/v aqueous sodium acetate) which formed a bright orange dye. Whatman No. 1 paper and upward solvent flow were used. The following R_F values were obtained: 0·59 with propan-1-ol–ammonia (7 : 3), 0·88 with butan-1-ol–acetic acid–water (3 : 1 : 1), 0·59 with ethyl methyl ketone–t-butyl alcohol–water–aq. ammonia (70 : 9 : 20 : 1) and 0·48 with butan-1-ol–ethanol–3N-ammonia (4 : 1 : 5).

1-*Benzoyl*-2-*naphthyloxyacetic Acid*.—1-Benzoyl-2-naphthol (5 g.; prepared from 2-naphthyl-benzoate by the Fries migration), chloroacetic acid (2·75 g.), sodium hydroxide (1·7 g.), and water (50 ml.) were heated at 100° for 9 hr. The cooled and acidified solution was extracted with ether, and the acidic product (1·5 g.) isolated by extraction of the ethereal solution with aqueous sodium hydrogen carbonate. This *acid* crystallised from aqueous methyl alcohol in prisms, m. p. 173—174° (with previous softening) (Found: C, 74·4; H, 4·7. $C_{19}H_{14}O_4$ requires C, 47·5; H, 4·6%). Attempted Baeyer–Villiger oxidation as described above for 2-acetyl-1-methoxynaphthalene gave only unchanged ketone, m. p. 169—170° undepressed on admixture with the starting material.

Baeyer–Villiger Oxidation of 1-*Acetyl*-2-*naphthyloxyacetic Acid*.—A solution of this naphthyloxyacetic acid (0·5 g.) and toluene-*p*-sulphonic acid (0·06 g.) in acetic acid (5 ml.) was treated with an 8·5% solution (14 ml.) of peracetic acid in acetic acid, set aside for 24 hr. at 35°, and poured into water. Extraction with ether gave an acid, m. p. 201—202° (Found: C, 61·8; H, 4·1. Calc. for $C_{10}H_8O_4$: C, 62·45; H, 4·2%), undepressed by admixture with 2-carboxycinnamic acid.

The authors thank Miss D. M. Fieldgate and Mr. D. R. Clifford for the microanalyses and Dr. J. F. W. McOmie for helpful discussion.

Department of Agriculture and Horticulture, University of Bristol,
Research Station, Long Ashton, Bristol. [*Received, September 14th*, 1962.]