The oligosaccharides formed have been compared chromatographically with the above trisaccharide and those components of the yeast invertase-sucrose reaction having similar R_F values. Conclusions are drawn about the transferase activity of banana invertase *in vivo*.

We are indebted to Dr A. Gottschalk of the Walter and Eliza Hall Institute of Research for his helpful discussions.

REFERENCES

- Allen, P. J. & Bacon, J. S. D. (1956). *Biochem. J.* **63**, 200.
 Bacon, J. S. D. (1954). *Biochem. J.* **57**, 320.
 Bacon, J. S. D. & Edelman, J. (1950). *Arch. Biochem.* **28**, 467.
 Bacon, J. S. D. & Edelman, J. (1951). *Biochem. J.* **48**, 114.
 Barnell, H. R. (1941). *Ann. Bot., N.S.*, **5**, 217.
 Bryson, J. L. & Mitchell, T. J. (1951). *Nature, Lond.*, **167**, 864.
 Edelman, J. (1956). *Advanc. Enzymol.* **17**, 189.
 Feingold, D. S., Avigad, G. & Hestrin, S. (1956). *Biochem. J.* **64**, 351.
 Folin, O. & Wu, H. (1920). *J. biol. Chem.* **41**, 367.
 Gottschalk, A. (1958). In *Handbuch der Pflanzenphysiologie*, vol. 2, p. 87. Ed. by Ruhland, W. Berlin: Springer-Verlag.
 Gottschalk, A. & Ada, G. L. (1956). *Biochem. J.* **62**, 681.
 King, E. J. (1951). In *Microanalysis in Medical Biochemistry*, 2nd ed., p. 23. London: J. and A. Churchill.
 Lane, J. H. & Eynon, L. (1950). In *Official and Tentative Methods of Analysis*, 7th ed., p. 506. Association of Official Agricultural Chemistry.
 Loesecke, H. W. von (1949). In *Bananas-Chemistry, Physiology, Technology*. New York: Interscience Publishers Inc.
 Mierau, F. (1893a). *Chemikerztg.* **17**, 1002.
 Mierau, F. (1893b). *Chemikerztg.* **17**, 1283.
 Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
 Pucher, G. W. & Vickery, H. B. (1936). *Industr. Engng Chem., (Anal.)*, **8**, 92.
 Raybin, H. W. (1933). *J. Amer. chem. Soc.* **55**, 2603.

Fungal detoxication

4. METABOLISM OF 2-METHOXYNAPHTHALENE BY *ASPERGILLUS NIGER**

By R. J. W. BYRDE, D. F. DOWNING AND D. WOODCOCK
Long Ashton Research Station, Bristol University

(Received 2 July 1958)

The metabolism of naphthalene by a variety of animals has been extensively studied by Young and by Boyland and their respective co-workers. The initial product in rats was shown by Young (1947) to be *D-trans*-1:2-dihydro-1:2-dihydroxynaphthalene, both the diol and 1-naphthol and their conjugates having been isolated from urine. There appears to be little breakdown of the naphthalene ring, however (Boyland & Wiltshire, 1953), which is in sharp contrast with the degradation of naphthalene compounds by soil micro-organisms (Walker & Wiltshire, 1953, 1955; Treccani, Walker & Wiltshire, 1954; Canonica, Fieocchi & Treccani, 1957).

In previous work by Byrde, Harris & Woodcock (1956) the metabolism of ω -(2-naphthyloxy)-*n*-alkylcarboxylic acids by *Aspergillus niger* van Tiegh. was studied, the main features being nuclear hydroxylation, together with β -oxidation of all side chains except those of 2-naphthyloxyacetic acid and β -(2-naphthyloxy)propionic acid. In a later paper (Byrde & Woodcock, 1958) a similar investigation, in which *Sclerotinia laxa* Aderh. & Ruhl. was used with the same series of acids, showed β -oxidation to be the main process involved,

nuclear hydroxylation being virtually absent. There was little evidence of ring breakdown of the naphthyloxy acid with either fungus, and the present investigations with 2-methoxynaphthalene as a non-polar sole-carbon source were made in order to examine the effect of this change on ring-fission.

EXPERIMENTAL

All m.p.'s are uncorrected. Ultraviolet-absorption spectra were determined in methanol solution with a Unicam SP. 500 spectrophotometer. Infrared-absorption spectra were determined on mulls prepared by intimately grinding the samples with Nujol, by using a Hilger 800 spectrophotometer.

MATERIALS

2-Methoxynaphthalene (nerolin). This was used as obtained from British Drug Houses Ltd., m.p. 73–74°.

2-Ethoxynaphthalene. This was prepared from 2-naphthol with diethyl sulphate and NaOH. Crystallized from ethanol, it had m.p. 35–36°.

4-Methoxysalicylic acid. This was prepared from 2:4-dihydroxybenzoic acid by the method of Gomberg & Johnson (1917). It crystallized from water in long silky needles, m.p. 157–158° (Found: C, 57.1; H, 4.8. Calc. for $C_8H_6O_4$: C, 57.1; H, 4.7%). Gomberg & Johnson (1917) give m.p. 157°. The ultraviolet-absorption spectrum showed

* Part 3 Byrde & Woodcock (1958).

the following maxima: 214 μ ($\epsilon_{\max}$, 18 780), 254 μ ($\epsilon_{\max}$, 12 260) and 295 μ ($\epsilon_{\max}$, 5590).

The infrared spectrum showed the following main absorption maxima (cm^{-1}): 2909, 1645, 1465, 1270.

4-Ethoxysalicylic acid. Prepared similarly to the corresponding methoxy compound, by using diethyl sulphate, this acid crystallized from water in silky white needles, m.p. 152–153° (Found: C, 59.1; H, 5.4. Calc. for $\text{C}_9\text{H}_{10}\text{O}_4$: C, 59.3; H, 5.5%). Perkin (1895) gives m.p. 153–154°. The infrared-absorption spectrum showed the following absorption maxima (cm^{-1}): 2932, 1655, 1465, 1275.

4-Methoxyphthalic acid. 4-Hydroxyphthalic acid, prepared by the method of Baeyer (1877), was methylated with dimethyl sulphate and NaOH. The methoxy acid crystallized from methanol in prisms, m.p. 165–166°. Bentley & Weizmann (1907) give m.p. 170°.

5-Amino-2-methoxynaphthalene. A solution of 2-methoxy-5-nitronaphthalene (1.8 g.), prepared as described by James & Woodcock (1951), in tetrahydrofuran (10 ml.) was shaken in hydrogen with Raney nickel; it rapidly absorbed the theoretical amount of gas. The blue fluorescent solution was filtered and evaporated under reduced pressure. The residual red oil was dissolved in ether and shaken with 10% (v/v) HCl; the acid solution was made alkaline with NaOH and the product was isolated with ether. The ethereal solution was dried (Na_2SO_4) and the solvent removed, leaving a red oil (1.1 g., yield 72%) which crystallized from aqueous methanol (charcoal) or light petroleum (b.p. 60–80°) in rosettes of prisms, m.p. 57–58° (Found: C, 76.5; H, 6.2; N, 8.2. Calc. for $\text{C}_{11}\text{H}_{11}\text{ON}$: C, 76.3; H, 6.3; N, 8.1%). On one occasion a dimorphic form, m.p. 73–74°, was obtained, and a solution of the lower-m.p. form gave the higher-m.p. form by seeding. Cohen, Cook, Hewett & Girard (1934) report needles, m.p. 73–74°; Campbell, La Forge & Campbell (1949) give m.p. 64–66°. **5-Acetamido-2-methoxynaphthalene** crystallized from water in clusters of needles, m.p. 124–125°, or from aqueous methanol in plates, m.p. 139–140° [Found (sample m.p. 124–125°): C, 72.8; H, 6.3; N, 6.6. Calc. for $\text{C}_{13}\text{H}_{15}\text{O}_2\text{N}$: C, 72.6; H, 6.1; N, 6.5%]. Cohen *et al.* (1934) give m.p. 142.5–143.5°.

5-Hydroxy-2-methoxynaphthalene. A solution of 5-amino-2-methoxynaphthalene (0.5 g.) in acetic acid (2.6 ml.) was stirred with H_2SO_4 (1.3 ml.) and water (1.3 ml.), and after cooling to 10° a solution of NaNO_2 (0.26 g.) in water (1 ml.) was added slowly dropwise. After stirring for 2 hr. the mixture was poured into boiling 5% (v/v) H_2SO_4 (80 ml.) and filtered hot, the filtrate cooled, extracted with ether and the ethereal layer shaken with 10% (w/v) NaOH. The alkaline washings were acidified with HCl and extracted with ether, and the ethereal extract dried (Na_2SO_4). Removal of the solvent gave a brown oil which crystallized from light petroleum (b.p. 40–60°) in colourless plates (0.35 g., 70%) m.p. 85° (Found: C, 76.0; H, 5.6. $\text{C}_{11}\text{H}_{10}\text{O}_2$ requires C, 75.9; H, 5.7%). This compound was reported as an oil, b.p. 185°/12 mm. Hg (Baeyer & Co., 1921).

6-Hydroxy-2-methoxynaphthalene. This was prepared as described by Downing & Woodcock (1958).

7-Hydroxy-2-methoxynaphthalene. This was prepared from 2,7-dihydroxynaphthalene according to the method of Fischer & Hammerschmidt (1916).

8-Nitro-2-methoxynaphthalene. 8-Nitro-2-naphthol (1.8 g.) (Friedlander & Szymanski, 1892) was methylated with dimethyl sulphate and NaOH. The oil obtained

crystallized from aqueous methanol in bright-yellow needles (1.5 g., 77%), m.p. 74–75° (Found: C, 65.2; H, 4.4; N, 7.1. Calc. for $\text{C}_{11}\text{H}_9\text{O}_2\text{N}$: C, 65.0; H, 4.4; N, 6.9%). Friedlander & Szymanski (1892) give m.p. 69°.

8-Amino-2-methoxynaphthalene. 2-Methoxy-8-nitronaphthalene (1 g.) was reduced as described for the 5-amino compound. The resultant red oil crystallized from aqueous methanol (charcoal) in prismatic plates (0.8 g., 89%), m.p. 79–80°. Bachmann & Horton (1947) give m.p. 79–80°.

8-Hydroxy-2-methoxynaphthalene. This was prepared from 8-amino-2-methoxynaphthalene as described for the 5-hydroxy compound. The red, oily product crystallized from light petroleum (b.p. 60–80°) as white plates (0.25 g., 50%), m.p. 100–102° (Found: C, 75.5; H, 5.6. Calc. for $\text{C}_{11}\text{H}_{10}\text{O}_2$: C, 75.9; H, 5.7%). Angeletti (1929) gives m.p. approx. 122–124°, after sintering at about 60°.

2-Carboxy-5-methoxycinnamic acid. This was prepared as described by Downing & Woodcock (1958).

Methods

Replacement-culture technique and chromatographic examination of metabolites. The general method followed that previously described (Byrde *et al.* 1956), except that the substrate was incubated with the mycelial mats of *A. niger* for periods of 1–7 days before being continuously extracted with ether. In view of the volatility of 2-methoxy- and 2-ethoxy-naphthalene, the amounts lost during incubation and extraction were estimated by including a flask containing substrate, but no fungus.

The chromatographic solvents used for examining these extracts were butan-1-ol-ethanol-3N- NH_3 (4:1:5, by vol.) (solvent A), aqueous 0.1N- NH_3 (solvent B) and benzene-propionic acid-water (10:7:1) (solvent C). Solvent B was useful for separation of naphthols which ran too near the solvent front in solvent A. After drying and exposure under ultraviolet light (wavelength of maximum emission 2539 Å), fluorescent spots were marked and the paper was then sprayed with either 2% aqueous FeCl_3 , a 0.02% solution of *p*-nitrobenzenediazonium fluoroborate (Freeman, 1952) in acetone, or B.D.H. universal indicator, which had been previously adjusted to pH 9–10 with NaOH.

Fungitoxicity in vitro. Comparisons of fungitoxicity were carried out by the method described by Byrde & Woodcock (1957), the medium being adjusted to approximately pH 2.0 after sterilization, by aseptic addition of H_3PO_4 (10%, v/v).

RESULTS

Metabolism of 2-methoxynaphthalene

Isolation of 4-methoxysalicylic acid. In a typical experiment in which 2-methoxynaphthalene (197.5 mg.) was incubated with *A. niger*, a total of 65 mg. was lost by volatilization in the incubator or recovered unchanged and 78 mg. of material soluble in aq. NaHCO_3 was obtained. Chromatographic examination of the acidic extract showed the presence of a strong acidic spot, R_F 0.59 (solvent A), R_F 0.69 (solvent B), R_F 0.78 (solvent C), which gave a violet colour with FeCl_3 and an orange-brown colour with *p*-nitrobenzenediazonium fluoroborate. The residue obtained by removal of the solvent from the ethereal extract

crystallized from water in fawn, felted needles, m.p. 156–157° (Found: C, 57.35; H, 4.7 %). The ultraviolet-absorption spectrum showed maxima at 214 m μ ($\epsilon_{\max}$ 20 030), 252 m μ ($\epsilon_{\max}$ 12 260) and 295 m μ ($\epsilon_{\max}$ 5410); the infrared spectrum showed main maxima (cm.⁻¹) as follows: 2932, 1655, 1465, 1270 m μ . This acid did not depress the m.p. of authentic 4-methoxysalicylic acid, and the ultraviolet- and infrared-absorption spectra are virtually identical. By chromatography only two weak spots were detectable in addition to that formed by 4-methoxysalicylic acid. The first, R_f 0.59 (i.e. coincident with 4-methoxysalicylic acid in solvent A) and R_f 0.74 (solvent C), gave an intense blue fluorescence under ultraviolet light (wavelength of maximum emission 2539 Å), whereas the second, R_f 0.84 (solvent A), which had a mauve fluorescence, appeared very similar to 7-methoxycoumarin, R_f 0.88 (solvent A). Attempts are being made to confirm its identity.

Attempted isolation of intermediates. The method of Walker & Wiltshire (1955) for the isolation of chloronaphthalenediol was modified as follows: The reaction mixture obtained after incubation for 24 hr. was stirred for 2 hr. with activated charcoal (1 g./l.); the charcoal was filtered off, dried in air and then extracted with a mixture of ethanol and benzene (1:1, v/v) in a Soxhlet apparatus for 8 hr. The solvents were removed under reduced pressure and the residue was treated with water and filtered and the aqueous solution was extracted repeatedly with ether. The combined extracts were washed with NaHCO₃, then with water, and were dried (Na₂SO₄) and the solvent was removed. The residual brown oil (X) did not crystallize from any of a variety of solvents. Chromatographic analysis indicated that it contained a phenolic compound (Y), R_f 0.48 (solvent B), which was different from any of the naphthols listed in Table 1.

Acidification and ether extraction of the NaHCO₃ washings gave 4-methoxysalicylic acid (identified chromatographically).

Metabolism of potential precursors of 4-methoxysalicylic acid. No 4-methoxysalicylic acid was detected when the following substrates (mM) were

incubated with *A. niger* for 7-day periods: 6-hydroxy-2-methoxynaphthalene, 4-methoxybenzoic acid, 4-methoxyphthalic acid and 2-carboxy-5-methoxycinnamic acid. The non-acidic fraction (X) obtained after incubation of 2-methoxynaphthalene with *A. niger* for 24 hr. also failed to yield 4-methoxysalicylic acid when incubated for 3 days with a fresh mycelial mat of *A. niger*. In another experiment, salicylic acid was not detected when *L-trans*-1:2-dihydro-1:2-dihydroxynaphthalene (kindly supplied by Dr N. Walker, Rothamsted Experimental Station) was incubated with *A. niger* for 2 days.

Metabolism of 2-ethoxynaphthalene

In a typical experiment in which 2-ethoxynaphthalene (215 mg.) was incubated with *A. niger*, a total of 46 mg. was lost by volatilization or recovered unchanged and 49 mg. of aq. NaHCO₃-soluble material was obtained. Chromatographic examination of the ethereal extract showed the presence of an acid, R_f 0.68 (solvent A), with the properties expected of 4-ethoxysalicylic acid. The residue obtained by removal of the solvent crystallized from aqueous methanol (charcoal) in needles, m.p. 152–153°, undepressed by admixture with authentic 4-ethoxysalicylic acid, m.p. 152–153°. The infrared-absorption spectrum, which showed maxima (cm.⁻¹) at 2932, 1655, 1465 and 1275, was identical with that of 4-ethoxysalicylic acid.

Fungitoxicity in vitro

Table 2 shows the mean growth rate (mm./day) at pH 2.0 of cultures of *A. niger* in the presence of 2-methoxynaphthalene, 2-ethoxynaphthalene and the corresponding salicylic acid detoxication products. Probit analysis of the quantitative data (Finney, 1952) is summarized in Table 3.

DISCUSSION

Although there was little evidence of ring-fission in the metabolism of ω -(2-naphthyloxy)-*n*-alkyl-carboxylic acids by *Aspergillus niger* and *Sclerotinia laxa* (Byrde *et al.* 1956; Byrde & Woodcock, 1958),

Table 1. *Chromatographic data of naphthols*

Compounds were run in aqueous 0.1N-NH₃ (solvent B) by downward solvent flow. After drying and exposure under ultraviolet light (wavelength of maximum emission, 2539 Å), fluorescent spots were marked and the paper was then sprayed with a 0.02% solution of *p*-nitrobenzenediazonium fluoroborate (Freeman, 1952) in acetone.

Compound	R_f	Fluorescence in u.v. light	Colour with diazo spray
Y	0.48	Blue	Deep pink
2-Naphthol	0.48	Purple	Salmon pink
5-Hydroxy-2-methoxynaphthalene	0.39	Deep blue	Light orange-brown
6-Hydroxy-2-methoxynaphthalene	0.28	Purple	Mauve-pink
7-Hydroxy-2-methoxynaphthalene	0.36	Purple	Light orange-brown
8-Hydroxy-2-methoxynaphthalene	0.43	Pale blue	Deep mauve-pink

this process is essential for the formation of 4-methoxysalicylic acid (II; $R = CH_3$), which is the only metabolic product of 2-methoxynaphthalene (I; $R = CH_3$) with *A. niger* which has been isolated.

Considering oxidative fission of 2-methoxynaphthalene, it would have seemed likely that the more active ring B should undergo initial attack,

Table 2. Mean growth rate of *A. niger* mycelium in the presence of 2-methoxynaphthalene, 2-ethoxynaphthalene and their detoxication products

Conditions were as described in the text.

Test no.	Compound added	Concn. (μM)	Mean rate of growth (mm./day)
1	2-Methoxynaphthalene	50	8.69
		100	8.00
		200	5.25
		400	2.56
		800	1.31
		1600	1.37
	4-Methoxysalicylic acid	70	13.00
		100	11.56
		140	8.37
		200	2.31
		280	0.00
		400	0.37
	Control	—	13.94
2	2-Ethoxynaphthalene	50	10.50
		100	10.00
		200	8.81
		400	6.00
		800	0.56
		1600	0.13
	4-Ethoxysalicylic acid	35	9.94
		50	8.31
		70	7.06
		100	4.75
		140	0.00
		200	0.00
	Control	—	11.44

but the isolation of 4-methoxysalicylic acid, together with the formation of 4-ethoxysalicylic acid (II; $R = C_2H_5$) from 2-ethoxynaphthalene (I; $R = C_2H_5$), implies that ring A has undergone degradation and was therefore more highly activated than ring B. The presence of the hydroxyl group in the final products suggests that this substitution must occur after ring-fission, since otherwise ring B would have been more active originally and hence degraded with the loss of the alkoxyl group. Hydroxylation after ring-fission is a feature of the scheme put forward by Fernley & Evans (1958) for the end-ring attack on naphthalene by soil *Pseudomonads*, the metabolic pathway to salicylic acid being by way of *D-trans*-1:2-dihydro-1:2-dihydroxynaphthalene, 1:2-dihydroxynaphthalene, *o*-carboxy-*cis*-cinnamic acid and *o*-hydroxy-cinnamic acid. A similar route via 2-hydroxy-4-methoxy-*cis*-cinnamic acid would seem to be the most likely for the formation of 4-methoxysalicylic acid from 2-methoxynaphthalene, and would explain the formation of 7-methoxycoumarin, the presence of which has been indicated chromatographically. An alternative pathway by way of 5:6-dihydro-5:6-dihydroxy-2-methoxynaphthalene, 5:6-dihydroxy-2-methoxynaphthalene and 2-carboxy-5-methoxycinnamic acid, which thus involves the ultimate replacement of $-CH:CH \cdot CO_2H$ by $-OH$, seems less likely, although the failure of 2-carboxy-5-methoxycinnamic acid to give 4-methoxysalicylic acid when incubated with *A. niger* could also be explained by stereospecificity of the fungal enzyme (cf. Evans & Smith, 1951). The possibility that 4-methoxyphthalic acid is involved in either route seems unlikely, since we were unable to detect the formation of 4-methoxysalicylic acid from 4-methoxyphthalic acid (cf. Henderson, 1957).

The successful isolation of a diol as a primary metabolic product has been reported from naphthalene (Walker & Wiltshire, 1953), 1-chloronaphthalene (Walker & Wiltshire, 1955) and 2-chloro- and 2-methyl-naphthalene (Canonica *et al.* 1957). The failure of Canonica *et al.* (1957) to isolate a diol intermediate from 1-methylnaphthalene by using *Pseudomonas desmolytica* has been followed by our

Table 3. Dose-response data derived from Table 2

Test no.	Compound	Equation of probit regression line	10^5 ED ₅₀ (M)	Slope of probit regression line
1	2-Methoxynaphthalene	$Y = 6.39 - 1.32x$	11.32 (2.58-21.52)	-1.32 ± 0.42
	4-Methoxysalicylic acid	$Y = 12.17 - 6.12x$	14.86 (10.32-18.52)	-6.12 ± 1.71
2	2-Ethoxynaphthalene	$Y = 9.98 - 3.16x$	37.58 (27.60-50.46)	-3.16 ± 0.55
	4-Ethoxysalicylic acid	$Y = 9.26 - 4.64x$	8.25 (6.60-9.73)	-4.64 ± 0.66

inability to isolate the expected diol from 2-methoxynaphthalene either as such or after conversion into hydroxymethoxynaphthalenes by acid treatment (cf. Walker & Wiltshire, 1955). Unlike Walker & Wiltshire (1953), Murphy & Stone (1955) found no evidence of the presence of *D-trans*-1:2-dihydro-1:2-dihydroxynaphthalene from a culture of a strain of *Pseudomonas* growing on naphthalene and concluded that possibly the 'diol' was utilized as rapidly as it was formed and consequently not detected in the medium. This is possibly what happens if a diol is formed from 2-methoxynaphthalene.

The toxicity studies were carried out at pH 2.0, where the salicylic acids would be expected to be present as undissociated molecules. The breakdown of 2-methoxynaphthalene to 4-methoxysalicylic acid was accompanied by some decrease in fungistatic activity to *A. niger* at the 50% response level. On the other hand, 4-ethoxysalicylic acid was more active than ethoxynaphthalene, and provides an example of a breakdown product (formed in substantial amount) which is more active than the initial material (cf. Williams, 1947). It seems likely that the low aqueous solubility of the two naphthalenes is a limiting factor in their fungistatic activity. In the corresponding salicylic acids, however, adequate solubility in water is assured by the presence of the polar groups, and an increase in the size of the lipophilic alkyloxy grouping would be expected to increase distribution to the biophase, and hence to increase fungistatic activity (Albert, 1951). It is also possible that chelation may play some part in the action of these acids at the higher pH values within the cell.

SUMMARY

1. By the use of a replacement-culture technique with *Aspergillus niger* 4-methoxysalicylic acid was isolated as a metabolic product from 2-methoxynaphthalene, and 4-ethoxysalicylic acid similarly from 2-ethoxynaphthalene.

2. 4-Methoxybenzoic acid, 4-methoxyphthalic acid, 2-carboxy-5-methoxycinnamic acid and 6-hydroxy-2-methoxynaphthalene did not produce 4-methoxysalicylic acid when incubated with *A. niger*.

3. Although the fungistatic activity (based on ED_{50} values at pH 2.0) of 4-methoxysalicylic acid was less than that of 2-methoxynaphthalene, 4-ethoxysalicylic acid was more active than the corresponding 2-ethoxynaphthalene.

The authors wish to thank Miss M. Waller and Miss N. Waugh for valuable technical assistance, Mr D. R. Clifford for the microanalyses and the ultraviolet spectra, and Dr E. R. Sayer for the infrared spectra.

REFERENCES

- Albert, A. (1951). *Selective Toxicity with Special Reference to Chemotherapy*. London: Methuen and Co. Ltd.
- Angeletti, A. (1929). *Gazz. chim. ital.* **59**, 851.
- Bachmann, W. E. & Horton, W. J. (1947). *J. Amer. chem. Soc.* **69**, 58.
- Baeyer, A. (1877). *Ber. dtsh. chem. Ges.* **10**, 1079.
- Baeyer and Co. (1921). *DRP 298 098*. Cited in *Chem. Zbl.* (1921), II, 504.
- Bentley, W. H. & Weizmann, Ch. (1907). *J. chem. Soc.* **91**, 98.
- Boyland, E. & Wiltshire, G. H. (1953). *Biochem. J.* **53**, 636.
- Byrde, R. J. W., Harris, J. F. & Woodcock, D. (1956). *Biochem. J.* **64**, 154.
- Byrde, R. J. W. & Woodcock, D. (1957). *Biochem. J.* **65**, 682.
- Byrde, R. J. W. & Woodcock, D. (1958). *Biochem. J.* **69**, 19.
- Campbell, K. N., La Forge, R. A. & Campbell, B. K. (1949). *J. org. Chem.* **14**, 346.
- Canonica, L., Fieocchi, A. & Treccani, V. (1957). *R.C. Ist. lombardo*, **91**, I, 119. Cited in *Chem. Abstr.* (1958). **52**, 12 988.
- Cohen, A., Cook, J. W., Hewett, C. L. & Girard, A. (1934). *J. chem. Soc.* p. 653.
- Downing, D. F. & Woodcock, D. (1958). *J. chem. Soc.* p. 531.
- Evans, W. C. & Smith, B. S. W. (1951). *Biochem. J.* **49**, x.
- Fernley, H. N. & Evans, W. C. (1958). *Nature, Lond.*, **182**, 373.
- Finney, D. J. (1952). *Probit Analysis*, p. 183. Cambridge University Press.
- Fischer, O. & Hammerschmidt, C. B. (1916). *J. prakt. Chem.* **94**, 24.
- Freeman, J. H. (1952). *Analyt. Chem.* **24**, 955.
- Friedlander, P. & Szymanski, St. (1892). *Ber. dtsh. chem. Ges.* **25**, 2079.
- Gomberg, M. & Johnson, L. C. (1917). *J. Amer. chem. Soc.* **39**, 1687.
- Henderson, M. E. K. (1957). *J. gen. Microbiol.* **16**, 686.
- James, P. M. & Woodcock, D. (1951). *J. chem. Soc.* p. 3418.
- Murphy, J. F. & Stone, R. W. (1955). *Canad. J. Microbiol.* **1**, 579.
- Perkin, A. G. (1895). *J. chem. Soc.* **67**, 995.
- Treccani, V., Walker, N. & Wiltshire, G. H. (1954). *J. gen. Microbiol.* **11**, 341.
- Walker, N. & Wiltshire, G. H. (1953). *J. gen. Microbiol.* **8**, 273.
- Walker, N. & Wiltshire, G. H. (1955). *J. gen. Microbiol.* **12**, 478.
- Williams, R. T. (1947). *Detoxication Mechanisms*, p. 241. London: Chapman and Hall Ltd.
- Young, L. (1947). *Biochem. J.* **41**, 417.