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THE CHEMISTRY OF MOULD METABOLITES—IV

REDUCTIVE ACETYLATION AND REOXIDATION OF SOME PHENOXAZIN-3-ONES

G. W. K. CAVILL, P. S. CLEZY and F. B. WHITFIELD
School of Chemistry, University of New South Wales, Australia

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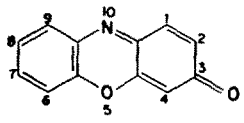
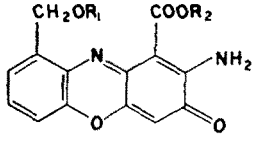
Abstract—The reductive acetylation of some substituted phenoxazin-3-ones including cinnabarinic acid is described, the products being characterized by infra-red spectroscopy. Additional support is provided for the structures assigned to the reduced acetylated derivatives of cinnabarin and its methyl ester. Nitrous acid has general application as an oxidant for the conversion of phenoxazines into phenoxazin-3-ones.

RECENT investigations have shown the existence of a new class of organic pigments in Nature represented by the ommochromes,[1] the actinomycins[2,3] and cinnabarin.[4,5] The presence of the phenoxazone chromophore (I) in these pigments has revived interest in phenoxazine chemistry, a study that has lain dormant for the last half century. The reductive acetylation of various phenoxazin-3-ones, and their subsequent reoxidation with nitrous acid has now been further investigated. These reactions have been employed in the characterization of cinnabarin and its derivatives.[4-6]

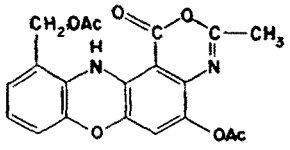


I

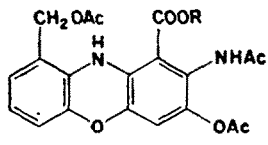


II

Reductive acetylation of cinnabarin (II, $R_1 = R_2 = H$) yielded triacetylanhydrodihydrocinnabarin (III)[4-6] whilst a triacetyldihydro derivative (IV, R = H) was obtained as a by-product.[5] Similarly, methyl cinnabarin (II, $R_1 = H$, $R_2 = CH_3$) gave methyl triacetyldihydrocinnabarin (IV, $R = CH_3$).[4,6] Since O-acetylcinnabarin (II, $R_1 = Ac$, $R_2 = H$) and methyl O-acetylcinnabarin (II, $R_1 = Ac$, $R_2 = CH_3$) can



III



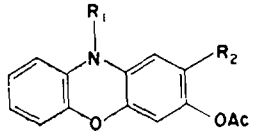
IV

[*] Part III, G. W. K. Cavill, P. S. Clezy, J. R. Tetaz and R. L. Werner, *Tetrahedron* **5**, 275 (1959).

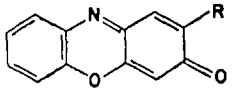
[1] A. Butenandt, V. Schieldt, E. Bickert and R. J. T. Cromartie, *Liebigs Ann.* **590**, 75 (1955).
[2] H. Brockmann and H. Muxfeldt, *Angew. Chem.* **68**, 69 (1956).
[3] S. J. Angyal, E. Bullock, W. G. Hangar, W. C. Howell and A. W. Johnson, *J. Chem. Soc.* 1592 (1957).
[4] G. W. K. Cavill, P. S. Clezy, J. R. Tetaz and R. L. Werner, *Tetrahedron* **5**, 275 (1959).
[5] J. Gripenberg, *Acta Chem. Scand.* **12**, 603 (1958).
[6] G. W. K. Cavill, B. J. Ralph, J. R. Tetaz and R. L. Werner, *J. Chem. Soc.* 525 (1953).

also be converted into III and IV (R = CH_3) respectively, one acetyl group was accommodated on the primary alcoholic group at position 9.[4,7] The second acetyl group was placed at position 3, thereby stabilizing the reduced products. The third was placed on the 2-amino substituent. The "anhydro" derivative (III) was formulated as an oxazinone, involving the cyclization of the 1-carboxy and 2-acetylamino substituents, and the absence of such a derivative from the products of reductive acetylation of methyl cinnabarin supports this formulation.[4,5]

In the present studies, the reductive acetylation of some model phenoxazin-3-ones gives two series of phenoxazine derivatives, in which, the degree of acetylation depends upon the reaction conditions. Under mild conditions, 3-acetoxyphenoxazines are obtained, whilst more vigorous conditions are required to achieve acetylation of the ring nitrogen atom. Mild reductive acetylation of phenoxazin-3-one (I) gives a monoacetyl derivative formulated as 3-acetoxyphenoxazine (V, $R_1 = R_2 = H$) on the basis of its absorption at 3336 cm^{-1} (NH, cf. phenoxazine 3386 cm^{-1}) and at 1750 cm^{-1} (aromatic acetate, cf. 3,5-dimethoxy-2-diacetylaminophenylacetate 1763 cm^{-1}).[8,*] The lowered NH frequency of 3-acetoxyphenoxazine is presumably due to hydrogen bonding with the ester group.[9] More vigorous reductive acetylation of phenoxazin-3-one gives a diacetyl derivative, transparent in the 3μ region of the spectrum, but showing absorption at 1756 cm^{-1} (aromatic acetate) and 1687 cm^{-1} (N-acetyl, cf. 10-acetylphenoxazine 1672 cm^{-1}). The diacetyl derivative is then formulated as 3-acetoxy-10-acetylphenoxazine (V, R_1 = Ac, R_2 = H). Comparably, 2-methoxy-3-acetoxyphenoxazine (V, R_1 = H, R_2 = OCH_3) and 2-methoxy-3-acetoxy-10-acetylphenoxazine (V, R_1 = Ac, R_2 = OCH_3) have been obtained from 2-methoxyphenoxazin-3-one (VI, R = OCH_3).



V



VI

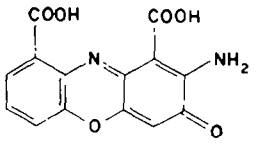
The pattern is complicated by the presence of the substituent groups in 2-hydroxy and 2-aminophenoxazin-3-one (VI, R = OH and R = NH_2, respectively). Vigorous reductive acetylation of 2-hydroxyphenoxazin-3-one produced a triacetate showing bands at 1775 cm^{-1} (aromatic acetate) and 1694 cm^{-1} (N-acetyl), which are consistent with structure (V, R_1 = Ac, R_2 = OAc) for this compound. The diacetate obtained under milder conditions, exhibits absorptions at 3342 cm^{-1} (NH) and 1767, 1749 cm^{-1} (aromatic acetate). The lower of these carbonyl absorptions is assigned to a hydrogen bonded ester carbonyl group. Mild reductive acetylation of 2-aminophenoxazin-3-one yields a diacetate, with absorptions at 3302 cm^{-1} (NH), 1730 cm^{-1} (aromatic acetate) and 1672 cm^{-1} (N-acetyl). Reference to the previous model compounds shows clearly that vigorous conditions are necessary for the acetylation of the bridge NH group, so that the latter frequency (1672 cm^{-1}) is assigned to an acetylamino group in position 2.

* Infra-red absorption spectra are reported for "Nujol" mulls. The majority of these acetylated product are insufficiently soluble for examination in solution.

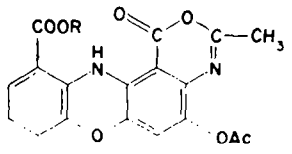
[7] J. Gripenberg, *Proc. Chem. Soc.* 233 (1957).
[8] J. F. Grove, P. W. Jeffs and D. W. Rustidge, *J. Chem. Soc.* 1956 (1956).
[9] R. E. Richards and H. W. Thompson, *J. Chem. Soc.* 1248 (1947).

Hence this diacetate is formulated as 2-acetylamino-3-acetoxyphenoxazine (V, $R_1 = H$, $R_2 = NHAc$). Two products were obtained by vigorous reductive acetylation of 2-aminophenoxazin-3-one, a triacetate (V, $R_1 = H$, $R_2 = NAc_2$) showing absorptions at 3340 cm^{-1} (NH), 1769 cm^{-1} (aromatic acetate), 1710, 1701 cm^{-1} (diacetylamine[8,10]), and a tetraacetate (V, $R_1 = Ac$, $R_2 = NAc_2$)[4] with absorptions at 1677 cm^{-1} (N-acetyl), 1770 cm^{-1} (aromatic acetate), and 1723, 1710 cm^{-1} (diacetylamine). These assignments are in accord with the formulations given above.

In contrast to these systems, cinnabarinic acid (VII) on reductive acetylation yields only one product, a diacetate, which has carbonyl bands at 1760 cm^{-1} and 1743 cm^{-1}



VII

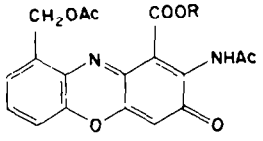


VIII

(aromatic acetate and oxazinone respectively, cf. triacetylanhydrodihydrocinnabarin 1769 and 1736 cm^{-1}). A third carbonyl band at 1672 cm^{-1} is assigned to the hydrogen bonded carbonyl group, and the absorption at 3240 cm^{-1} is that of the N—H group of the phenoxazine ring. Diacetylanhydrodihydrocinnabarinic acid is thus formulated as (VIII, R = H), and methylation of this compound with diazomethane, yields a monomethyl ester (VIII, $R = CH_3$) further supporting the presence of the oxazinone system.

The above results verify the structures III and IV, ($R = CH_3$) proposed independently[4,5] for the reduced acetylated derivatives of cinnabarin and of methyl cinnabarin. Moreover, the isolation of only one derivative from the reductive acetylation of cinnabarinic acid supports the hypothesis[4,5] that acetylation of the ring nitrogen atom is prevented by steric effects of the large groups at positions 1 and 9.

Previous papers[4,11] from this laboratory have described the use of nitrous acid for the oxidation of triacetylanhydrodihydrocinnabarin (III) to O, N-diacetylcinnabarin (IX, R = H), and for the oxidation of methyl triacetyldihydrocinnabarin (IV, $R = CH_3$) to methyl O-N-diacetylcinnabarin (IX, $R = CH_3$).



IX

This oxidation process is now shown to have general application. Phenoxazine is converted into phenoxazin-3-one (I), at 5°, but more vigorous conditions (60° for 5 mins) are needed to obtain the same product from 10-acetylphenoxazine. 3-Acetoxyphenoxazine (V, $R_1 = R_2 = H$) and 3-acetoxy-10-acetylphenoxazine (V, $R_1 = Ac$, $R_2 = H$) also yield phenoxazin-3-one, when treated with nitrous acid at 5°. Similarly, 2-acetylamino-, 2-diacetylamino-, and 10-acetyl-2-diacetylamino-3-acetoxyphenoxazine (V, $R_1 = H$, $R_2 = NHAc$; $R_1 = H$, $R_2 = NAc_2$; $R_1 = Ac$, $R_2 = NAc_2$

[10] R. A. Abramovitch, *J. Chem. Soc.* 1413 (1957).
[11] G. W. K. Cavill, P. S. Clezy and J. R. Tetaz, *J. Chem. Soc.* 2646 (1957).

respectively) are converted into 2-acetylaminophenoxazin-3-one (VI, R = NHAc); whilst 2,3 diacetoxyphenoxazine (V, R_1 = H, R_2 = OAc) and 2,3-diacetoxy-10-acetylphenoxazine (V, R_1 = Ac, R_2 = OAc) yield 2-acetoxyphenoxazin-3-one (VI, R = OAc). Finally, 2-methoxy-3-acetoxyphenoxazine (V, R_1 = H, R_2 = OCH_3) and its 10-acetyl derivative, require a more vigorous oxidation to give 2-methoxyphenoxazin-3-one (VI, R = OCH_3).

The generality of the oxidation process described above, confirms the phenoxazin-3-one structures (IX, R = H and R = CH_3) previously assigned[4] to the products obtained by the action of nitrous acid on triacetylanhydrodihydrocinnabarin (III) and methyl triacetyldihydrocinnabarin (IV, R = CH_3).

EXPERIMENTAL

Light petroleum has b.p. 60–80°. Alumina refers to aluminium oxide, grade H, (Peter Spence), neutralized by the method given previously.[11] Carbon, hydrogen and nitrogen analyses are by Dr. E. Challen of this University, additional analyses are by C.S.I.R.O. Microanalytical Laboratory (Melbourne). Wenzel's dilute sulphuric acid was used for hydrolysis during acetyl determinations. The infra-red absorption spectra, determined by Mr. I. H. Reece, were measured in "Nujol", and the bands in the 3μ and 6μ regions are recorded below. Mr. D. Weedon determinated the ultra-violet absorption spectra using 95% ethanol as solvent. All m.p.s are uncorrected.

3-Acetoxyphenoxazine. Phenoxazin-3-one (750 mg), prepared after Kehrmann and Saager,[12] and purified by alumina chromatography and sublimation *in vacuo*, in acetic anhydride (20 ml) and pyridine (1 ml) was shaken with an excess of zinc dust at room temp for 15 min and then heated at at 100° for 3 min. The pale yellow solution, separated from excess zinc by decantation, was poured onto ice when a white solid precipitated (300 mg). This solid was dissolved in chloroform, the solution washed with saturated aqueous sodium hydrogen carbonate, and water, and then dried (Na_2SO_4). Evaporation of the solvent gave a crystalline residue, which after recrystallization from benzene–light petroleum gave *3-acetoxyphenoxazine*, as colourless plates, m.p. 144–145°. ν_{max} 3336 (m), 1750 (s) cm^{-1}. (Found: C, 70·1; H, 4·3; N, 5·75; Ac, 18·4%. $C_{14}H_{11}O_3N$ requires: C, 70·0; H, 4·2; N, 5·8; Ac, 17·9%).

10-Acetyl-3-acetoxyphenoxazine. Phenoxazin-3-one (500 mg), in acetic anhydride (30 ml) and pyridine (2 ml) was shaken with zinc dust (4 g) at room temp for 10 min. The pale yellow reaction mixture, after heating under reflux for 10 min, was worked up as above.

10-Acetyl-3-acetoxyphenoxazine (250 mg) was obtained from benzene–light petroleum as colourless needles, m.p. 124·5—126°. ν_{max} 1756 (s), 1687 (s) cm^{-1}. (Found: C, 67·6; H, 4·35; N, 5·0; Ac, 30·0%. $C_{16}H_{13}O_4N$ requires: C, 67·8; H, 4·6; N, 4·95; 2Ac, 30·4%).

2-Hydroxyphenoxazin-3-one (modification of Diepolder's method).[13] A mixture of 2-aminophenol (6 g), ferric chloride hexahydrate (40 g) and hydrochloric acid (10 N; 350 ml) was heated under reflux for 6 hr. The deep brown solution was diluted with water (700 ml), extracted with chloroform (16 × 100 ml) and the latter extract shaken with aqueous sodium carbonate (5%; 6 × 200 ml). The green fluorescent chloroform solution was rejected. The deep red alkaline solution was acidified to give a yellow precipitate (2 g) which was purified by vacuum sublimation (170–200°/2 mm), to give 2-hydroxyphenoxazin-3-one as a bright orange solid, m.p. 264° (decomp). Diepolder[13] reports m.p. 278° (decomp). (Found: C, 67·5; H, 3·4; N, 6·5%. Calc. for $C_{12}H_7O_3N$: C, 67·4, H, 3·3; N, 6·6%).

2,3-Diacetoxyphenoxazine. 2-Hydroxyphenoxazin-3-one (500 mg), in acetic anhydride (22 ml) and pyridine (1 ml) was heated under reflux for 3 min. When cool, the red solution was shaken with zinc dust (2 g) at room temp for 10 min, and then heated at 100° for 3 min. By the usual procedure, 2,3-diacetoxyphenoxazine (650 mg) was obtained as colourless plates, m.p. 145-146° from benzene–light petroleum. ν_{max} 3342 (w), 1767 (s), 1749 (s) cm^{-1}. (Found: C, 64·5; H, 4·4; N, 4·7; Ac, 28·0%. $C_{16}H_{13}O_5N$ requires: C, 64·2; H, 4·4; N, 4·7; 2Ac, 28·8%).

10-Acetyl-2,3-diacetoxyphenoxazine. (a) 2-Hydroxyphenoxazin-3-one (150 mg), in acetic anhydride (6 ml) and pyridine (3 drops) was heated under reflux for 3 min. The red solution, after

[12] F. Kehrmann and A. Saager, *Ber. Dtsch. Chem. Ges.* **35**, 341 (1902).
[13] E. Diepolder, *Ber. Dtsch. Chem. Ges.* **35**, 2816 (1902).

cooling, was shaken with zinc dust (200 mg) at room temp for 10 min. The resultant colourless solution was heated under reflux (1 hr) whence 10-*acetyl*-2-3-*diacetoxyphenoxazine* (120 mg) was finally obtained, as colourless plates, m.p. 142·5–143°, from benzene–light petroleum, depressed to 114° on admixture with 2,3-diacetoxyphenoxazine. ν_{max} 1775 (s) 1694 (s) cm^{-1} (Found: C, 63·0; H, 4·15; N, 4·55; Ac, 36·2%. $C_{18}H_{15}O_6N$ requires: C, 63·35; H, 4·4; N, 4·1; 3Ac, 37·7%).

(b) 2,3-Diacetoxyphenoxazine (450 mg) in acetic anhydride (15 ml) and pyridine (0·5 ml) was heated under reflux for 30 min. By the usual procedure, 10-*acetyl*-2,3-*diacetoxyphenoxazine* (300 mg) was obtained as colourless plates m.p. 142·5–143° from benzene–light petroleum. (Found: C, 63·6; H, 4·4; N, 4·4%. $C_{18}H_{15}O_6N$ requires: C, 63·35; H, 4·4; N, 4·1%).

2-*Methoxyphenoxazin*-3-*one*. 2-Hydroxyphenoxazin-3-one (500 mg) and anhydrous potassium carbonate (13·4 g) were suspended in dry acetone (500 ml) containing methyl iodide (10 g). The mixture was heated under reflux for 5 hr when methyl iodide (10 g) was added, and the heating continued (5 hr). The potassium carbonate was removed by filtration and the filtrate reduced in volume (100 ml) *in vacuo*, whereupon 2-*methoxyphenoxazin*-3-*one* (280 mg) precipitated. A further quantity (50 mg) was obtained by alumina chromatography of the residue obtained by complete removal of the solvent. Purification, by sublimation (180–190°/1·0 mm), gave 2-methoxyphenoxazin-3-one as a pale yellow solid, m.p. 255° (decomp). λ_{max} 222, 395 mμ (ε, 23·7 × 10^3, 18·1 × 10^3). (Found: C, 68·5; H, 3·8; N, 5·8% $C_{13}H_9O_3N$ requires: C, 68·7; H, 4·0; N, 6·2%).

2-*Methoxy*-3-*acetoxyphenoxazine*. 2-Methoxyphenoxazin-3-one (150 mg), in acetic anhydride (5 ml) and pyridine (2 drops) was shaken with zinc dust (150 mg) at room temp for 15 min. The colourless solution, separated from the excess zinc by decantation, was poured into ice, and the resultant solid was purified in the usual way. 2-Methoxy-3-acetoxyphenoxazine (150 mg) was obtained from benzene–light petroleum as colourless needles, m.p. 172–174°. ν_{max} 3336 (m), 1756 (s) cm^{-1}. (Found: C, 66·65; H, 5·0; N, 5·1; Ac, 14·3%. $C_{15}H_{13}O_4N$ requires: C, 66·4; H, 4·8; N, 5·2; Ac, 15·85%).

10-*Acetyl*-2-*methoxy*-3-*acetoxyphenoxazine*. 2-Methoxyphenoxazin-3-one (300 mg), in acetic anhydride (12 ml) and pyridine (1 ml) was shaken with zinc dust (3 g) at room temp for 10 min, after which the colourless solution was heated under reflux for 10 min. 10-*Acetyl*-2-*methoxy*-3-*acetoxyphenoxazine* (400 mg) was obtained from aqueous alcohol as colourless needles, m.p. 148–149°. ν_{max} 1755 (s), 1681 (s) cm^{-1}. (Found: C, 65·4; H, 4·9; N, 4·4; Ac, 25·3%. $C_{17}H_{15}O_5N$ requires: C, 65·2; H, 4·8; N, 4·5; 2Ac, 27·1%).

2-*Aminophenoxazin*-3-*one* (after Fischer and Jonas).[14] 2-Aminophenol (5 g) in chloroform (300 ml), was treated at 60° with yellow mercuric oxide (15 g) added over 15 min, the mixture being well stirred. Finally, the mixture was heated under reflux for 10 min, the deep red solution filtered and the filtrate concentrated *in vacuo* (50 ml). 2-Aminophenoxazin-3-one (4·7 g) which was obtained in a crystalline state from the concentrate, was further purified by recrystallization from benzene, to give deep purple plates, m.p. 256–257° (decomp) Fischer and Jonas[14] report m.p. 249° (decomp). (Found: C, 68·0; H, 3·6; N, 13·05%. Calc. for $C_{12}H_8O_2N_2$: C, 67·95; H, 3·8; N, 13·2%).

2-*Acetylamino*-3-*acetoxyphenoxazine*. 2-Aminophenoxazin-3-one (1 g), in acetic anhydride (43 ml), and pyridine (2 ml), was shaken with zinc dust (4 g) at room temp for 1½ hr. The colourless reaction mixture was then heated at 100° for 5 min. The usual working up procedure gave 2-*acetylamino*-3-*acetoxyphenoxazine* (900 mg) as colourless needles m.p. 220° from chloroform–benzene. ν_{max} 3302 (s), 1730 (s), 1675 (s) cm^{-1}. (Found: C, 64·3; H, 4·3; N, 9·1; Ac, 27·5%. $C_{16}H_{14}O_4N_2$ requires: C, 64·4; H, 4·7; N, 9·4; 2Ac, 28·9%).

10-*Acetyl*-2-*diacetylamino*-3-*acetoxyphenoxazine*. (a) 2-Aminophenoxazin-3-one (1 g), in acetic anhydride (43 ml) and pyridine (2 ml) was shaken with zinc dust (4 g) at room temp for 10 min, and the colourless reaction mixture then heated under reflux for 10 min. Following the usual procedure, the reduced acetylated product was obtained as a colourless solid, further resolved on recrystallization from benzene. The more soluble fraction, when recrystallized from benzene–light petroleum gave 10-acetyl-2-diacetylamino-3-acetoxyphenoxazine (600 mg) as heavy colourless plates, m.p. 181–182°. Cavill *et al*[4] reports m.p. 184–185°. ν_{max} 1677 (m), 1710 (s), 1723 (s), 1770 (s) cm^{-1}. (Found: C, 62·8; H, 4·6; N, 7·2; Ac, 45·1%). Calc. for $C_{20}H_{18}O_6N_2$: C, 62·75; H, 4·75; N, 7·3; 4Ac, 45·1%).

The less soluble product gave 2-*diacetylamino*-3-*acetoxyphenoxazine* (150 mg) further purified by

[14] O. Fischer and O. Jonas, *Ber. Dtsch. Chem. Ges.* 27, 2782 (1894).

recrystallization from benzene–light petroleum as fine colourless needles m.p. 195–196°. ν_{max} 3340 (m), 1769 (s), 1710 (s), 1701 (s) cm^{-1}. (Found: C, 63·9; H, 4·4; N, 8·0; Ac, 37·4%. $C_{18}H_{16}O_6N_2$ requires: C, 63·5; H, 4·75; N, 8·2; 3Ac, 38·0%).

(b) 2-Acetylamino-3-acetoxyphenoxazine (60 mg), in acetic anhydride (5 ml) and pyridine (3 drops), was heated under reflux for 10 min, to yield 10-acetyl-2-diacetylamino-3-acetoxyphenoxazine (60 mg), which was isolated as colourless plates from benzene–light petroleum, m.p. and mixed m.p. with an authentic specimen, 179–180°. (Found: C, 63·1; H, 4·6; N, 7·4%. Calc. for $C_{20}H_{18}O_6N_2$: C, 62·75, H, 4·75; N, 7·3%).

(c) 2-Diacetylamino-3-acetoxyphenoxazine (70 mg), in acetic anhydride (6 ml) and pyridine (0·5 ml) was heated under reflux for 15 min. 10-Acetyl-2-diacetylamino-3-acetoxyphenoxazine (80 mg) was obtained as colourless plates, m.p. and mixed m.p. 181–182°, from benzene–light petroleum. (Found: C, 63·1; H, 4·8; N, 7·2%. Calc. for $C_{20}H_{18}O_6N_2$: C, 62·75; H, 4·75; N, 7·3%).

Diacetylanhydrodihydrocinnabarinic acid. Cinnabarinic acid[15] (175 mg), in acetic anhydride (10 ml) and pyridine (0·5 ml) was shaken with zinc dust (500 mg) at room temp for 5 min, and then heated at 100° for 5 min. *Diacetylanhydrodihydrocinnabarinic acid* (170 mg) was obtained as yellow needles, m.p. > 300° (decomp), from dioxan. λ_{max} 228, 245, 427–434 mμ (ε, 30·2 × 10^3, 22·8 × 10^3 12·2 × 10^3). ν_{max} 3240 (w), 1677 (s) 1743 (s), 1760 (s) cm^{-1}. (Found: C, 58·8; H, 3·4; N, 7·8; Ac, 20·8%. $C_{18}H_{12}O_7N_2$ requires C, 58·7; H, 3·3; N, 7·6; 2Ac, 23·4%). The identical product was obtained when cinnabarinic acid was reductively acetylated by heating under reflux for 10 min.

Diacetylanhydrodihydrocinnabarinic acid monomethyl ester. Diazomethane (2·3 g in 100 ml ether) was added to a suspension of diacetylanhydrodihydrocinnabarinic acid (160 mg) in ether (1 l.), and the mixture allowed to stand at 5° for 24 hr, then at room temp overnight. Dilute N acetic acid (7 ml) was added to destroy excess diazomethane, and the ethereal solution evaporated to dryness. Repeated recrystallization of the residue from benzene–light petroleum gave *diacetylanhydrodihydrocinnabarinic acid monomethyl ester* (40 mg) as yellow needles, m.p. 255–259° (Found: C, 60·2; H, 4·1; N, 7·2; Ac, 17·8%. $C_{19}H_{14}O_7N_2$ requires: C, 59·8; H, 3·7; N, 7·3; Ac, 22·5%).

Oxidation of phenoxazine. Phenoxazine (100 mg) in ethanol (95%; 5 ml) and 10N HCl (1 ml) was treated at 5° with aqueous sodium nitrite (30%; 2 ml). The colour of the reaction solution changed from red to dark blue and warming to 60° affected no change. After standing for 3 days at room temp the green reaction solution was extracted with chloroform (2 × 20 ml), the organic phase washed with saturated aqueous sodium hydrogen carbonate and water, and dried (Na_2SO_4). Removal of the solvent gave an orange solid (50 mg) which was purified by alumina chromatography, and sublimation (140–160°/1 mm) to give phenoxazin-3-one, m.p. 205–207° (decomp), undepressed on admixture with an authentic specimen. λ_{max} 243, 347, 447 mμ (ε, 17·6 × 10^3, 12·4 × 10^3, 11·1 × 10^3). (Found: C, 73·6; H, 3·8; N, 7·2%. Calc. for $C_{12}H_7O_2N$: C, 73·1; H, 3·6; N, 7·1%.)

Oxidation of 10-acetylphenoxazine. 10-Acetylphenoxazine (30 mg) dissolved in ethanol (95% 10 ml) and 10N HCl (3 ml) was treated at 5° with aqueous sodium nitrite solution (30%; 5 ml). No visible reaction was evident. The temp was then raised to 60° for 5 min, brown fumes were evolved and the solution became deep orange. After allowing the reaction mixture to stand at room temp; for 1 day, it was extracted with chloroform and worked up by the method outlined for the previous oxidation. Phenoxazin-3-one (5 mg) was isolated after vacuum sublimation, m.p. and mixed m.p. 208–209° (decomp). λ_{max} 245, 348, 447 mμ (ε, 18·5 × 10^3, 13·3 × 10^3, 11·3 × 10^3).

Oxidation of 3-acetoxyphenoxazine. 3-Acetoxyphenoxazine (100 mg), in ethanol (95%; 5 ml) and 10N HCl (1 ml) was treated at 5° with aqueous sodium nitrite solution (30%; 3 ml) for 3 days. By the above procedure phenoxazin-3-one (80 mg) was obtained after vacuum sublimation (140–160°/1 mm), m.p. and mixed m.p. 205–206° (decomp). λ_{max} 245, 348, 447 mμ (ε, 16·5 × 10^3, 12·1 × 10^3, 11·1 × 10^3). (Found: C, 72·8; H, 3·55, N, 7·3%. Calc. for $C_{12}H_7O_2N$: C, 73·1; H, 3·6; N, 7·1%).

Oxidation of 10-acetyl-3-acetoxyphenoxazine. 10-Acetyl-3-acetoxyphenoxazine (100 mg), in ethanol (95%; 5 ml) and 10 N HCl (1 ml) was treated for 3 days at 5° with aqueous sodium nitrite solution (30%; 3 ml). Phenoxazin-3-one (70 mg) was isolated, m.p. and mixed m.p. 206–208° (decomp). λ_{max} 245, 348, 447 mμ (ε, 17·6 × 10^3, 12·8 × 10^3, 11·1 × 10^3). (Found: C, 72·6; H, 3·4; N, 7·3%. Calc. for $C_{12}H_7O_2N$: C, 73·1; H, 3·6; N, 7·1%).

Oxidation of 2-acetylamino-3-acetoxyphenoxazine. 2-Acetylamino-3-acetoxyphenoxazine (150 mg), in ethanol (95%; 20 ml) and 10N HCl (3 ml) was treated at 5° for 2 hr with aqueous sodium

[15] A. Butenandt, J. Keck and G. Neubert, *Liebigs Ann.* **602**, 61 (1957).

nitrate (30%; 10 ml). A yellow solid (135 mg) deposited, which after purification by alumina chromatography and recrystallization from benzene, gave 2-acetylaminophenoxazin-3-one as yellow needles m.p. 274° (decomp) undepressed by an authentic sample prepared after Fischer and Hepp.[16] λ_{max} 240, 400 mμ (ε, 32·6 × 10³, 25·9 × 10³). (Found: C, 65·9; H, 3·9; N, 10·95%. Calc. for $C_{14}H_{10}O_3N_2$ C, 66·1; H, 4·0; N, 11·0%).

Oxidation of 2-diacetylamino-3-acetoxyphenoxazine. 2-Diacetylamino-3-acetoxyphenoxazine (100 mg), in ethanol (95%; 10 ml) and 10N HCl (1 ml) was treated at 5° for 2 hr with aqueous sodium nitrite (30%; 3 ml). Chloroform extraction of this solution gave an orange product which was purified as above to give 2-acetylaminophenoxazin-3-one, m.p. and mixed m.p. 290° (decomp). λ_{max} 240, 400 mμ (ε, 31·4 × 10³, 24·8 × 10³). (Found: C, 66·3; H, 4·1; N, 11·3%. Calc. for $C_{14}H_{10}O_3N_2$: C, 66·1; H, 4·0; N, 11·0%).

Oxidation of 10-acetyl-2-diacetylamino-3-acetoxyphenoxazine. 10-Acetyl-2-diacetylamino-3-acetoxyphenoxazine (150 mg), in ethanol (95%; 15 ml) and 10N HCl (3 ml) was treated at 5° for 1 hr with aqueous sodium nitrite solution (30%; 8 ml). The precipitated orange solid (120 mg) was purified by recrystallization from benzene. The less soluble fraction yielded 2-acetylaminophenoxazin-3-one (50 mg) m.p. and mixed m.p. 274° (decomp). λ_{max} 240, 400 mμ (ε, 32·6 × 10³, 26·2 × 10³). (Found: C, 66·2; H, 3·9, N, 10·7%. Calc. for $C_{14}H_{10}O_3N_2$: C, 66·1; H, 4·0; N, 11·0%).

The more soluble fraction gave 10-acetyl-2-diacetylamino-3-acetoxyphenoxazine (38 mg), m.p. and mixed m.p. 178–179°. (Found: C, 62·9; H, 4·7; N, 7·5%. Calc. for $C_{20}H_{18}O_6N_2$: C, 62·75; H, 4·75; N, 7·3%).

Oxidation of 2,3-diacetoxyphenoxazine. 2,3-Diacetoxyphenoxazine (200 mg), in dioxan (10 ml) and 10N HCl (1 ml) was treated overnight at 5° with aqueous sodium nitrite solution (30%; 4 ml). 2-Acetoxyphenoxazin-3-one (70 mg) was obtained by the usual procedure as red leaflets, m.p. 229–230° (decomp), from benzene–light petroleum. Its m.p. was not depressed by an authentic sample prepared after Diepolder.[13] λ_{max} 245, 363, 448 mμ (ε, 15·2 × 10³, 13·0 × 10³, 9·1 × 10³). (Found: C, 66·0; H, 3·7; N, 5·5%. Calc. for $C_{14}H_9O_4N$: C, 66·0; H. 3·6; N, 5·5%).

Oxidation of 10-acetyl-2,3-diacetoxyphenoxazine. 10-Acetyl-2,3-diacetoxyphenoxazine (100 mg) in dioxan (8 ml) and 10N HCl (1 ml) was treated at 5° for 36 hr with aqueous sodium nitrite (30% 2 ml). The precipitated red solid gave 2-acetoxyphenoxazin-3-one (50 mg), after recrystallization from benzene–light petroleum, m.p. and mixed m.p. 229–230° (decomp). λ_{max} 245, 359, 447 mμ (ε, 15·2 × 10³, 12·8 × 10³, 9·0 × 10³). (Found: C, 65·9; H, 3·55; N, 5·8%. Calc. for $C_{14}H_9O_4N$: C, 66·0; H, 3·6; N, 5·5%).

Oxidation of 2-methoxy-3-acetoxyphenoxazine. 2-Methoxy-3-acetoxyphenoxazine (90 mg), in ethanol (95%; 5 ml) and 10N HCl (1 ml) was treated with aqueous sodium nitrite solution (30%; 3 ml). After no apparent reaction occurred at 5° during 3 hr, the reaction mixture was heated at 60° for 5 min. Brown fumes were liberated and the deep orange reaction mixture was allowed to stand at 5° for 2 days. 2-Methoxyphenoxazin-3-one (50 mg) was obtained and purified by sublimation (160–180°/1·0 mm), m.p. and mixed m.p. 255–257°. λ_{max} 222, 390 mμ (ε, 23·1 × 10³, 17·9 × 10³). (Found: C, 68·4, H, 4·0; N, 6·1%. Calc. for $C_{13}H_9O_3N$: C, 68·7; H, 4·0; N, 6·2%).

Oxidation of 10-acetyl-2-methoxy-3-acetoxyphenoxazine. 10-Acetyl-2-methoxy-3-acetoxyphenoxazine (70 mg), in ethanol (95%; 5 ml) and 10N HCl (1 ml) was treated with aqueous sodium nitrite solution (30%; 2 ml) at 60° for 5 min and then at 5° for 4 days. 2-Methoxyphenoxazin-3-one (40 mg) was isolated, which after vacuum sublimation (160–180°/1 mm) had m.p. and mixed m.p. 255–257° (decomp). λ_{max} 221, 395 mμ (ε, 24·4 × 10³, 17·2 × 10³). (Found: C, 68·5; H, 3·9; N, 6·15%. Calc. for $C_{13}H_9O_3N$: C, 68·7; H, 4·0; N, 6·2%).

[16] O. Fischer and E. Hepp, *Ber. Dtsch. Chem. Ges.* **28**, 293 (1895).