Tetrahedron, 1959, Vol. 6, pp. 331 to 337. Pergamon Press Ltd. Printed in Northern Ireland

ANTHRAQUINONE AND ANTHRONE SERIES—XXIII*

THE NON-IDENTITY OF 1:3:8-TRIHYDROXY-2-HYDROXYMETHYL-ANTHRAQUINONE WITH VERSICOLORIN AND A SYNTHESIS OF DAMNACANTHOL AND DAMNACANTHAL

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(*Received* 9 *December* 1958)

Abstract—1:3:8-Trihydroxy-2-hydroxymethylanthraquinone has been synthesised by hydroxymethylation of 1:3:8-trihydroxyanthraquinone and shown to be different from versicolorin, the colouring matter isolated by Hatsuda and Kuyama from *Aspergillus versicolor*. Damnacanthol and damnacanthal, the colouring matters of the roots of *Damnacanthus major* and *D. indicus* have been synthesised by a new route.

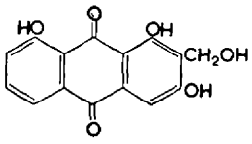
FROM the mycelium of *Aspergillus versicolor* (Vuillemin) Tiraboschi, cultured on a malt extract medium containing glucose and peptone, Hatsuda and Kuyama[1] isolated two colouring matters, versicolorin, yellow-orange needles, m.p. 282°, and sterigmatocystin, yellow needles, m.p. 243°. Hatsuda *et al.*[2] ascribed to sterigmatocystin the molecular formula $C_{15}H_{12}O_5$ and a structure derived from xanthhydrol. This pigment was isolated later from the same organism by Davies *et al.*[3] and by Birkinshaw and Hammady.[4] The latter authors found the molecular formula of sterigmatocystin to be $C_{18}H_{12}O_6$, which excluded both the molecular and structural formulae proposed by Hatsuda. Birkinshaw and Hammady also obtained a second optically active pigment which crystallised from chloroform in long orange needles, m.p. 233–234°. Although colour reactions and ultra-violet absorption spectra indicated it to be a polyhydroxyanthraquinone, the H : C ratio was too high. The colouring matter was not identical with versicolorin which had a different melting point and for which no optical activity was reported.

Versicolorin has been isolated from *A. versicolor* only by the Japanese workers, and they have assigned the molecular formula $C_{15}H_{10}O_6$ and the structure I or II. Evidence for the hydroxyanthraquinone structure was a purple colour in aqueous sodium hydroxide, which disappeared on heating with zinc dust and was restored by air oxidation. With alcoholic magnesium acetate versicolorin gave an orange solution, indicating that two hydroxyl groups were probably located *meta* to each other.[5] The formation of a tetra-acetate and a trimethyl ether by heating with dimethyl sulphate, potassium carbonate and acetone showed the presence of one alcoholic and three phenolic hydroxyl groups. By analogy with other fungal anthraquinones two hydroxyls were placed in 1:8-positions, the three phenolic hydroxyls therefore being

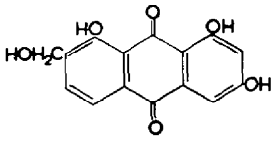
* Part XXII: *Tetrahedron* **3**, 62 (1958).

[1] Y. Hatsuda and S. Kuyama, *J. Agr. Chem. Soc. Japan* **28**, 989 (1954).
[2] Y. Hatsuda, S. Kuyama and N. Terashima, *J. Agr. Chem. Soc. Japan* **28**, 992, 998 (1954); **29**, 11 (1955).
[3] J. E. Davies, J. C. Roberts and S. C. Wallwork, *Chem. & Ind.* 178 (1956).
[4] J. H. Birkinshaw and I. M. M. Hammady, *Biochem. J.* **65**, 162 (1957).
[5] S. Shibata, M. Takido and O. Tanaka, *J. Amer. Chem. Soc.* **72**, 2789 (1950).

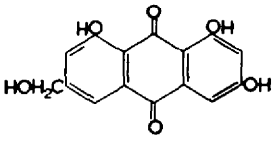
in the 1:3:8-positions. The 6-position for the hydroxymethyl group was ruled out, since versicolorin would then be identical with citreorosein (III: ω-hydroxyemodin). On the assumption that the hydroxymethyl group was in a β-position, based on the fact that no naturally occurring anthraquinone colouring matter with a hydroxymethyl group in an α-position has so far been encountered, it followed that versicolorin was constituted as I or II.



I



II



III

1:3:8-Trihydroxy-2-hydroxymethylanthraquinone (I) has now been synthesised by an unambiguous method, and several of its properties are different from those described for versicolorin, a sample of which was kindly supplied by Professor Hatsuda. Versicolorin therefore does not have the structure I. We have shown

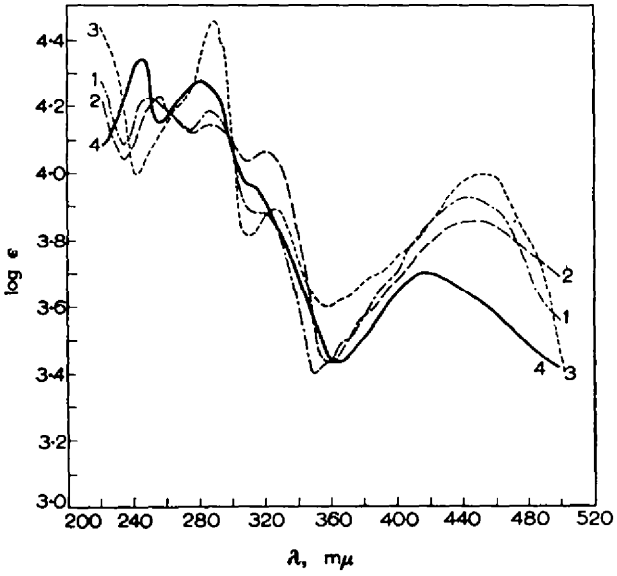


FIG. 1. Solvent—ethanol.

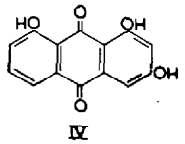
Absorption spectra of:
1. — — — 1:3:8-Trihydroxyanthraquinone
2. ——— 1:3:8-Trihydroxy-2-hydroxymethylanthraquinone
3. - - - - Versicolorin
4. ——— Lucidin (1:3-dihydroxy-2-hydroxymethylanthraquinone)

earlier that lucidin (2-hydroxymethylxanthopurpurin) can be readily synthesised by the condensation of xanthopurpurin (1:3-dihydroxyanthraquinone) with formaldehyde and sodium hydroxide solution.[6] Likewise, hydroxymethylation of 1:3:8-trihydroxyanthraquinone (IV) gave an excellent yield of I, which darkens and decomposes at 295°; the m.p. quoted for versicolorin is 282°. The tetra-acetates of I and versicolorin melt at 205° and 225° respectively.

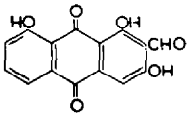
[6] N. R. Ayyangar and K. Venkataraman, *J. Sci. Ind. Res.* **B15**, 359 (1956).

Versicolorin and the synthetic compound I differ in their absorption spectra in the ultra-violet and visible regions (Fig. 1; lucidin and 1:3:8-trihydroxyanthraquinone are included for comparison). The infra-red spectrum of I (KBr pellet) shows absorption bands at 3436 cm^{-1} (alcoholic and β-hydroxyl groups, which are not distinguishable), 1670 cm^{-1} (unchelated carbonyl) and 1617 cm^{-1} (chelated carbonyl). In the region above 1600 cm^{-1} the following maxima have been recorded[7] for lucidin (1:3-dihydroxy-2-hydroxymethylanthraquinone): 3448, 3367, 1667 and 1621 cm^{-1}. Versicolorin shows absorption bands at 3330 cm^{-1}, 1673 cm^{-1} (very weak band seen as an inflexion or shoulder), and 1620 cm^{-1}. Reviewing Hatsuda's work on versicolorin, Thomson[8] has stated that "as there appears to be only one carbonyl band in the infra-red spectrum these (structures I and II) must be accepted with reserve," but there are two carbonyl bands in the infra-red spectrum of the sample of versicolorin sent to us by Professor Hatsuda. When the infra-red spectra were determined in Nujol, the chelated carbonyl band of both I and versicolorin was overlapped by the phenyl band and a strong absorption band was observed around 1604–1606 cm^{-1}. This type of overlapping of the phenyl and chelated carbonyl bands has been observed in the infra-red spectra of 1:5- and 1:8-dihydroxyanthraquinones determined by the Nujol mull technique.[9] The possibility of versicolorin having the structure II cannot be eliminated on the available evidence, and the synthesis of II is in progress.

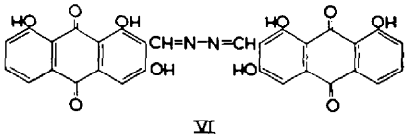
Oxidation of I with manganese dioxide in boiling benzene gave the corresponding aldehyde V, which is of interest on account of the occurrence of other hydroxyanthraquinone aldehydes, damnacanthal (see below) and fallacinal,[10] in nature. Two molecules of the aldehyde (V) reacted with hydrazine to give the compound (VI), establishing that the aldehyde group in V occupies a β-position and that IV therefore had undergone hydroxymethylation in the 2-position yielding I. If IV had undergone hydroxymethylation in the 4-position, the corresponding aldehyde would have reacted only with one molecule of hydrazine to form a pyridazine derivative.



IV



V

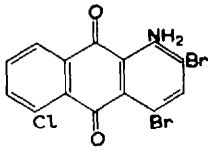


VI

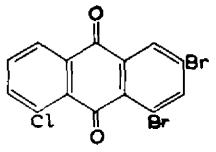
A synthesis of 1:3:8-trihydroxyanthraquinone (IV) starting from 1-amino-6:8-dichloroanthraquinone was reported earlier,[11] but the following route is more convenient. Bromination of 1-amino-5-chloroanthraquinone gave 1-amino-2:4-dibromo-5-chloroanthraquinone (VII). Deamination of VII by diazotization and boiling with ethanol gave the trihalogenoanthraquinone (VIII), which was converted to 1:3:8-trimethoxyanthraquinone by refluxing with sodium methoxide and copper oxide in methanol. The replacement of both the α- and β-halogens by methoxyl groups was facilitated by the use of copper oxide. When VIII was heated with calcium hydroxide and copper bronze under pressure, only the α-halogens were replaced by hydroxyl

[7] L. H. Briggs and G. A. Nicholls, *J. Chem. Soc.* 3068 (1953).
[8] R. H. Thomson, *Naturally Occurring Quinones* p. 206. Butterworths, London (1957).
[9] O. Tanaka, *Pharm. Bull. Japan* **6**, 18 (1958).
[10] T. Murakami, *Pharm. Bull. Japan* **4**, 298 (1956).
[11] N. Parkash and K. Venkataraman. *J. Sci. Ind. Res.* **B13**, 825 (1954).

groups. Demethylation of the trimethyl ether by means of aluminium chloride–sodium chloride at 145–150° then yielded 1:3:8-trihydroxyanthraquinone (IV).

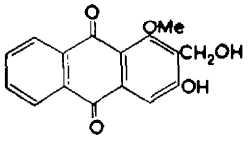


VII

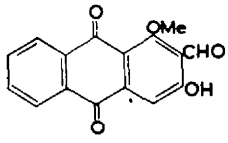


VIII

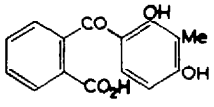
Nonomura[12] isolated two colouring matters, damnacanthol (IX) and damnacanthal (X), from the roots of the Japanese *Rubiaceae Damnacanthus major* Sieb and Zucc., *D. major* Sieb and Zucc. var. *parvifolius* Koidz., and *D. indicus* Gaertner fil. var. *microphyllus* Makino. He indicated the close similarity between damnacanthal (X) and the compound $C_{16}H_{10}O_5$, m.p. 208°, isolated by Perkin and Hummel[13] from *Morinda umbellata* Linn. While the present work was in progress, Hirose[14] reported the synthesis of IX and X, starting from the very difficultly accessible resorcinol



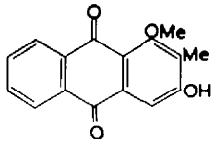
IX



X



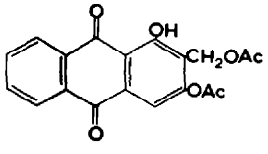
XI



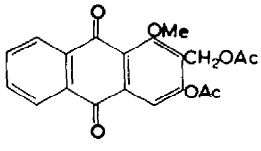
XII

derivative (XI). The keto-acid (XI) was cyclized to rubiadin, from which the 1-methyl ether (XII) was prepared and submitted to the action of N-bromosuccinimide, following our route for the synthesis of lucidin from rubiadin.[15] As mentioned earlier, lucidin has become readily available by the direct hydroxymethylation of xanthopurpurin, and the preparation of damnacanthol (IX) and damnacanthal (X) from lucidin is now described.

Lucidin-2:3-diacetate (XIII) was prepared by heating a solution of lucidin, boroacetic anhydride and acetic anhydride, and treating the diboroacetate thus



XIII



XIV

[12] S. Nonomura, *J. Pharm. Soc. Japan* **75**, 219, 222, 225 (1955).
[13] A. G. Perkin and J. J. Hummel, *J. Chem. Soc.* **65**, 854 (1894).
[14] Y. Hirose, *J. Pharm. Soc. Japan* **76**, 1448 (1956).
[15] B. S. Joshi, N. Parkash and K. Venkataraman, *J. Sci. Ind. Res.* **B14**, 87 (1955).

formed with water; XIII could also be obtained by acetylating lucidin with acetic anhydride and potassium acetate at 15–20°. Methylation of XIII with diazomethane to 1-methoxy-2-acetoxymethyl-3-acetoxyanthraquinone (XIV) and subsequent hydrolysis with methanolic potassium hydroxide gave damnacanthol (IX), m.p. 295° (dec). The alcohol (IX) on oxidation with manganese dioxide in boiling benzene gave X, m.p. 211°, undepressed on admixture with a sample of natural damnacanthal kindly supplied by Professor Nonomura. The identity of the synthetic compound (X) and natural damnacanthal was also shown by the ultra-violet and infra-red spectra.

EXPERIMENTAL

1-Amino-2:4-dibromo-5-chloroanthraquinone (VII). To a stirred mixture of 1-amino-5-chloroanthraquinone (20 g) and glacial acetic acid (800 ml) at 100°, bromine (20 ml) in glacial acetic acid (40 ml) was added drop by drop in 2 hr. Agitation was continued at 100° for 5 hr, and the dark red product was then collected, washed with 2 per cent sodium bisulphite solution and water, and dried (26 g). Crystallisation from chlorobenzene gave dark red needles, m.p. 250° (Found: C, 40·5; H, 1·9; N, 3·3; Cl, 8·7; Br, 39·0. $C_{14}H_6O_2Br_2ClN$ requires: C, 40·5; H, 1·5; N, 3·4; Cl, 8·4; Br, 38·6%).

1:3-Dibromo-8-chloroanthraquinone (VIII). 1-Amino-2:4-dibromo-5-chloroanthraquinone (25 g) was dissolved in conc H_2SO_4 (250 ml), cooled in an ice-bath to 5°, and diazotised with sodium nitrite (20 g) in conc H_2SO_4 (100 ml) for 1 hr. Glacial acetic acid (50 ml) was added, and after 15 min, the mixture was poured over crushed ice (1 kg). The solution of the diazonium salt was added to ethanol (1 l.) and the mixture was gradually heated to the boil and then refluxed for 30 min. The greyish yellow product was collected, washed free of acid and dried (22 g). Crystallisation from glacial acetic acid gave greyish yellow needles, m.p. 245° (Found: C, 41·6; H, 1·2. $C_{14}H_5O_2Br_2Cl$ requires: C, 42·0; H, 1·3%).

3-Bromo-1:8-dihydroxyanthraquinone. A mixture of 1:3-dibromo-8-chloroanthraquinone (VIII; 5 g), calcium hydroxide (30 g), copper bronze (2 g) and water (100 ml) was heated in a rocking autoclave at 230° and 27 atmospheres pressure for 24 hr. The violet calcium salt was acidified with 5% HCl, the brown product collected, washed, and extracted with 5% NaOH, and the extract acidified. The precipitate was collected, washed free of acid and dried (2·6 g). On extraction with petroleum ether, an orange yellow product (2 g) was obtained, which on crystallisation from glacial acetic acid gave orange-yellow needles m.p. 210° (Found: C, 53·2; H, 2·1; Br, 25·1. $C_{14}H_7O_4Br$ requires: C, 52·7; H, 2·2; Br, 25·1%).

The *diacetyl* derivative crystallised from glacial acetic acid in pale yellow needles, m.p. 192° (Found: C, 53·7; H, 2·4; Br, 20·2. $C_{18}H_{11}O_6Br$ requires: C, 53·6; H, 2·7; Br, 19·8%).

1:3:8-Trimethoxyanthraquinone. 1:3-Dibromo-8-chloroanthraquinone (3 g) and copper oxide (1 g) were refluxed for 24 hr with sodium methoxide solution prepared by reacting sodium (7·5 g) with dry methanol (150 ml). The mixture was then poured into water (500 ml), and the yellow product was collected, washed and dried (1·95 g). It crystallised from ethanol in yellow platelets, m.p. 196°.[11] (Found: C, 68·8; H, 4·8; OMe, 29·9. $C_{17}H_{14}O_5$ requires: C, 68·5; H, 4·8; OMe, 31·2%).

1:3:8-Trihydroxyanthraquinone (IV). 1:3:8-Trimethoxyanthraquinone (1·7 g) was added to a melt of anhydrous aluminium chloride (8 g) and sodium chloride (1·5 g) at 145–150°. The mixture was stirred at 150° for 5 min and then poured into 5% HCl (150 ml). The brown-red precipitate was coagulated by heating for a few min, collected (1·5 g), and crystallised from ethyl acetate; the brown plates melted at 287°.[11] (Found: C, 65·5; H, 3·1. $C_{14}H_8O_5$ requires: C, 65·6; H, 3·1%). The substance dissolves in conc H_2SO_4 with a reddish orange colour and gives a red solution in aqueous caustic soda and sodium carbonate. These properties are in agreement with those described.[11] The infra-red spectrum (KBr pellet) shows maxima at 3330, 1670, 1617, 1582, 1480, 1454, 1416, 1368, 1338, 1280, 1230, 1163, 1056, 1020, 973, 913, 881, 863, 831, 821, 807, 778, 759, 741 and 724 cm^{-1}.

1:3:8-Trihydroxy-2-hydroxymethylanthraquinone (I). A solution of 1:3:8-trihydroxyanthraquinone (1·5 g) in 5% NaOH (20 ml) was cooled to 20–25°, and treated with formalin (37–40%; 0·6 ml) under stirring. After 30 min agitation at 20–25°, the mixture was allowed to stand for 16 hr, then acidified, and the golden yellow precipitate collected, washed and dried (1·5 g). The product, which was very sparingly soluble in ethyl acetate and ethanol, crystallised from dioxan in orange yellow

microscopic needles, darkening and decomposing above 295°. (Found: C, 62·8; H, 3·8. $C_{16}H_{10}O_6$ requires: C, 62·9; H, 3·7%). The substance gives a red solution in sodium carbonate and sodium hydroxide solutions and a brown solution in H_2SO_4. Versicolorin gives a violet solution in sodium carbonate and sodium hydroxide solutions and a bright violet solution in H_2SO_4. The infra-red spectrum shows maxima at 3436, 2940, 1670, 1617, 1572, 1480, 1454, 1416, 1368, 1338, 1309, 1280, 1254, 1218, 1196, 1163, 1039, 996, 974, 916, 897, 877, 859, 838, 807, 793, 765 and 730 cm^{-1}. Versicolorin shows maxima at 3330, 2940, 1670, 1620, 1582, 1484, 1449, 1382, 1313, 1284, 1236, 1218, 1182, 1163, 1064, 1044, 1030, 996, 970, 940, 904, 874, 857, 824, 781, 761, 742 and 723 cm^{-1}.

The *tetracetyl* derivative, prepared in the usual manner by means of acetic anhydride and pyridine, crystallised from ethanol in pale yellow needles, m.p. 205° (Found: C, 60·4; H, 4·0. $C_{23}H_{16}O_{10}$ requires: C, 60·8; H, 4·0%).

1:3:8-*Trimethoxy-2-hydroxymethylanthraquinone.* A mixture of 1:3:8-trihydroxy-2-hydroxymethylanthraquinone (0·14 g), anhydrous potassium carbonate (3 g), dimethyl sulphate (0·25 ml), and acetone (100 ml) was refluxed on a water-bath for 24 hr. After removal of the acetone and dilution of the mixture with water, a yellow product was obtained, which crystallised from ethanol in shining yellow needles, m.p. 220° (Found: C, 65·9; H, 4·7. $C_{18}H_{16}O_6$ requires: C, 65·9; H, 4·9%).

1:3:8-*Trihydroxyanthraquinone-2-aldehyde* (V). 1:3:8-Trihydroxy-2-hydroxymethylanthraquinone (IV, 0·4 g) and activated manganese dioxide[16] (0·8 g) were refluxed with benzene (150 ml) for 16 hr. The mixture was filtered hot, and the manganese dioxide residue washed several times with hot benzene. Evaporation of the solvent and crystallisation of the residue from glacial acetic acid gave brownish yellow needles (0·25 g), m.p. 223° (Found: C, 63·4; H, 3·0. $C_{15}H_8O_6$ requires: C, 63·4; H, 2·8%). The aldehyde dissolves in warm sodium hydroxide solution with a red-violet colour, and in conc H_2SO_4 with an orange-red colour. The *azine* (VI) was prepared by treating a warm solution of V (0·01 g) in glacial acetic acid (5 ml) with excess of hydrazine hydrate solution. Immediately a flocculent yellow crystalline precipitate separated. Crystallisation from nitrobenzene gave yellow needles, m.p. >360°. (Found: N, 5·0. $C_{30}H_{16}O_{10}N_2$ requires: N, 5·0%).

Lucidin-2:3-diacetate (XIII). (a) Lucidin[6] (2 g) was refluxed with a mixture of boroacetic anhydride (5 g) and acetic anhydride (25 ml) for 5 min. The crystalline diboroacetate which separated was filtered, washed with ice-cold acetic anhydride containing boroacetic anhydride and finally with ether. The diboroacetate was suspended in water (100 ml) for 12 hr at room temp. The product (2 g) gave on crystallisation from ethanol yellow needles, m.p. 152° (Found: C, 64·4; H, 3·9. $C_{19}H_{14}O_7$ requires: C, 64·2; H, 3·9%). An ethanolic solution of XIII gives a brown colour with alcoholic ferric chloride.

(b) A mixture of lucidin (1·5 g), acetic anhydride (10 ml) and potassium acetate (1 g) was agitated at 15–20° for one hr and kept overnight. It was then poured into ice-cold water (100 ml) and the product (1·6 g) collected. Crystallisation from ethanol gave yellow needles, m.p. 152°, identical with the diacetate obtained by method (a).

3-*Acetoxy-2-acetoxymethyl-1-methoxyanthraquinone* (XIV). To a solution of lucidin-2:3-diacetate (3 g) in tetrachloroethane (40 ml) excess of ethereal diazomethane was added under stirring. After 48 hr excess of diazomethane was destroyed by acetic acid, ether distilled off, and tetrachloroethane removed by steam distillation; the residue crystallised from ethanol in yellow needles (1·5 g), m.p. 156–157° (Found: C, 65·3; H, 4·5; OMe, 8·1. $C_{20}H_{16}O_7$ requires: C, 65·2; H, 4·3; OMe, 8·4%).

Lucidin-1-methylether (*Damnacanthol;* IX). A solution of XIV (1·5 g) in 5% methanolic potassium hydroxide (50 ml) was maintained at 25° for 24 hr. Acidification with acetic acid gave a bright yellow crystalline product (0·75 g) which crystallised from ethanol in yellow needles, m.p. 295° (with darkening) (Found: C, 67·5; H, 4·2; OMe, 11·1. $C_{16}H_{12}O_5$ requires: C, 67·5; H, 4·2; OMe, 10·9%).

3-*Hydroxy-1-methoxyanthraquinone-2-aldehyde* (*Damnacanthal;* X). Lucidin-1-methyl ether (0·5 g) was refluxed with manganese dioxide[16] and benzene (40 ml) for 24 hr, and the solution filtered hot. The manganese dioxide residue was washed several times with hot benzene. After removal of the benzene, the residue (0·3 g) crystallised from ethanol in orange-yellow needles, m.p. 211°, undepressed on admixture with a sample of natural damnacanthal kindly supplied by Prof. Nonomura (Found C, 68·4; H, 3·6. $C_{16}H_{10}O_5$ requires: C, 68·1; H, 3·6%). The infra-red spectra of damnacanthal and the compound (X) in carbon tetrachloride show maxima at 2935, 1965, 1876, 1754, 1670, 1610, 1570, 1510, 1468, 1382, 1354, 1270, 1220, 1194, 1064, 1030 and 982 cm^{-1}. In the ultra-violet and

[16] M. Harfenist, A. Bavley and W. A. Lazier, *J. Org. Chem.* **19**, 1608 (1954).

visible regions both damnacanthal and X exhibit the following maxima: 2500, 2810, 3800 and 6150 Å; log ε_{max} 4·40, 4·37, 3·60 and 3·20 respectively.

The 2:4-*dinitrophenylhydrazone* of X crystallised from nitrobenzene in golden yellow needles, m.p. 300° (Found: N, 12·3. $C_{21}H_{12}O_8N_4$ requires: N, 12·1%).

Acknowledgements—We are indebted to Professor Hatsuda for a sample of versicolorin, to Professor Nonomura for a sample of damnacanthal, to the Council of Scientific and Industrial Research for the award of a fellowship to one of us (N. R. A.), to Dr. G. D. Shah and Mr. V. S. Pansare for the microanalyses recorded in the paper, and to Dr. (Mrs.) S. Dasgupta for the infra-red spectra.