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SHORT COMMUNICATION

3-METHYLPURPURIN AND OTHER ANTHRAQUINONES FROM CALLUS TISSUE OF *DIGITALIS LANATA**

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Abstract—Four additional anthraquinones, one of them new, have been isolated from the callus tissue of *Digitalis lanata*, from which 4-hydroxydigitolutein (V) and digitolutein (VI) have previously been obtained. The new pigment is 3-methylpurpurin (III) and the others are 3-methylquinizarin (I), pachybasin (II) and 3-methylalizarin (IV). This is the first report of I, II, III and IV as metabolic products of plant callus tissues.

INTRODUCTION

AS REPORTED in an earlier paper,[1] six anthraquinones (the pigments Q_1–Q_6) have been isolated from the callus tissue of *Digitalis lanata* (Scrophulariaceae) and major pigments Q_5 and Q_6 determined as 4-hydroxydigitolutein (V) and digitolutein (VI), respectively. The structural elucidation of minor pigments Q_1–Q_4 are described in this paper and the occurrence of these pigments in the callus and in the mother plant has also been compared.

RESULTS AND DISCUSSION

The acidic and neutral fractions obtained from methanolic extracts of fresh callus tissue of *Digitalis lanata* were chromatographed over a silica gel column and eluted with benzene.

The first pigment Q_1 from the benzene eluate of the acidic fraction gave orange–red needles, m.p. 175–178°, $C_{15}H_{10}O_4$ and its diacetate m.p. 217–219°, $C_{19}H_{14}O_6$. Q_1 was identified as 3-methylquinizarin (I) by UV, IR, mass and NMR, and by comparison with a synthetic sample. Although 3-methylquinizarin (I) has already been isolated from heartwood of *Tectona grandis*[2] (Verbenaceae), this is the first report of its occurrence in plant callus tissue.

The second yellow pigment Q_2, $C_{15}H_{10}O_3$ isolated from the acidic fraction and in higher yield from the neutral fraction, melted at 176–177°. The IR and mass spectra indicated that it was either 1-hydroxy-3-methyl- or 1-hydroxy-2-methylanthraquinone. Direct comparison by IR and mixed m.p. with synthetic compounds showed that it was pachybasin (1-hydroxy-3-methylanthraquinone) (II). II has previously been isolated from *Pachybasium candidum*,[3] *Phoma foveata*[4] and other fungi,[5] but this is only the second report of it in higher plants.†

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* Part XIV in the series "Studies in Plant Tissue Cultures." For part XIII see K. SYONO and T. FURUYA, *Experientia* in press.

† The isolation of pachybasin from *Tectona grandis* (Verbenaceae) is reported in Ref. 5 as an unpublished observation of the author.

[1] T. FURUYA and H. KOJIMA, *Phytochem.* **10**, 1607 (1971).

[2] W. SANDERMANN and M. H. SIMATUPANG, *Naturwissenschaften* **52**, 262 (1965).

[3] S. SHIBATA and M. TAKIDO, *Chem. Pharm. Bull. Tokyo* **3**, 156 (1955).

[4] I. R. C. BICK and C. RHEE, *Biochem. J.* **98**, 112 (1966).

[5] R. H. THOMSON, *Naturally Occurring Quinones* (2nd Edition), p. 372, Academic Press, New York (1971).

The third pigment Q_3 was obtained in small amount as brown–red needles, $C_{15}H_{10}O_5$, which gave a pink color with 2 N NaOH. The presence of two absorption maxima,[6] 484 and 519 nm, in the visible region and the TLC behavior suggested that it was a purpurin-like compound. It was then identified as 3-methylpurpurin (III) by R_f, mass and UV spectral comparison with synthetic material. This is the first report of it as a natural product.

The fourth pigment Q_4 afforded orange needles, m.p. 249–250°, $C_{15}H_{10}O_4$, and gave a diacetate, light yellow needles, m.p. 212–213°, $C_{19}H_{14}O_6$. From IR, UV and MS data, behavior of TLC and GLC and comparison with a synthetic sample, it was identified as 3-methylalizarin (IV). IV has already been isolated from *Digitalis purpurea*,[7] but not from plant tissue culture.

It is of interest from the biosynthetic viewpoint (Fig. 1) that I, II, III and IV together with V and VI have substituents on only one of the benzenoid rings and could thus be derived from mevalonic acid.[8]

I, II and VI were also detected by TLC and GLC in the aerial parts and the root of the mother plant, from which the callus tissue was derived. V was possibly present as well. This is the first report of I and II as natural constituents of *Digitalis* species.

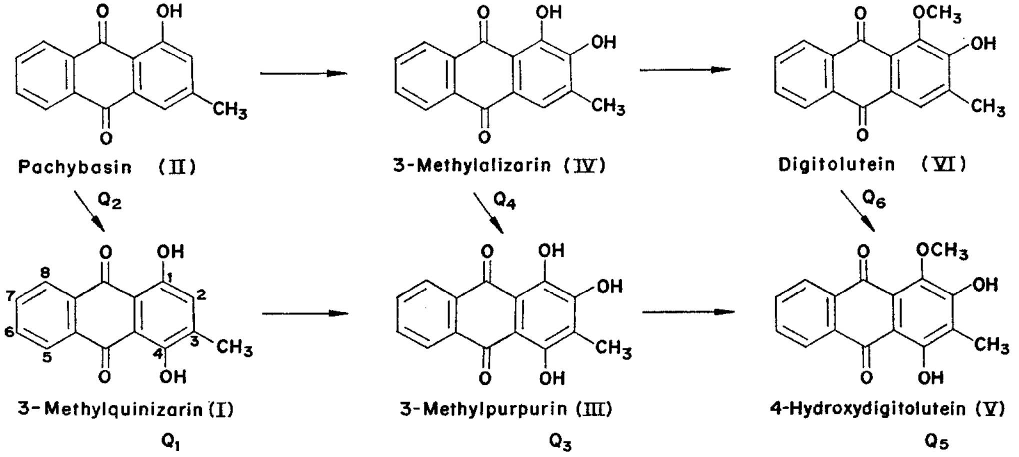

FIG. 1. POSSIBLE SCHEME FOR THE BIOSYNTHESIS OF ANTHRAQUINONES FOUND IN CALLUS TISSUE OF *D. lanata*

EXPERIMENTAL

M.p's were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were recorded on JNN-4H-100 spectrometer and peak positions are given in δ values with tetramethylsilane as an internal standard. Mass spectra were obtained on a JMS-OIS spectrometer with a direct inlet. Molecular formulae were mainly measured by mass spectrometer.

Plant Tissue Culture

The callus derived from seedling of *D. lanata* in February, 1965 was subcultured on Murashige and Skoog's basal medium containing IAA 1 ppm and kinetin 0·1 ppm in the same way as described previously.[1]

Isolation of Anthraquinone Pigments from Callus Tissue

The callus (fresh wt. 3 kg) harvested after culturing for 8–10 weeks was homogenized with cold MeOH

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[6] T. IKEDA, Y. YAMAMOTO, K. TSUKIDA and S. KANATOMO, *J. Pharm. Soc. Japan* **76**, 217 (1956).

[7] A. R. BURNETT and R. H. THOMSON, *Phytochem.* **7**, 1423 (1968).

[8] E. LEISTNER and M. H. ZENK, *Tetrahedron Letters* 1395 (1968).

in Waring blender, refluxed and filtered. The filtrate was evaporated to a small volume *in vacuo*. The concentrated aqueous solution was extracted with C_6H_6 and the C_6H_6 solution was shaken with 2 N NaOH. The alkali solution after acidification was again shaken with C_6H_6. Each neutral and acidic fraction after drying (Na_2SO_4) was evaporated to dryness. The acidic and neutral fractions, respectively, were chromatographed on column of deactivated silica gel (Kanto Kagaku) and then eluted with C_6H_6 [each fraction; 50 ml (in the acidic fraction) and 100 ml (in the neutral)].

3-*Methylquinizarin* (I). Fraction 7–8 (from the acidic fraction) was concentrated to red pigment Q_1 which upon recrystallization from MeOH, gave orange-red needles (I, 2·5 mg), m.p. 175–178° and mixed m.p. 176–178°, $C_{15}H_{10}O_4$ (Calc. for 254·058) 254·057, UV λ_{max} (EtOH) 226 (sh) (log ϵ 4·42), 233 (4·42), 251 (4·70), 257 (sh) (4·63), 286 (4·13), 330 (sh) (3·45), 460 (sh) (3·89), 472 (sh) (3·91), 485 (3·94), 506 (sh) (3·78), 519 (3·73) nm. IR ν_{max} (KBr) 1623 (chelated C=O), 1582 (C=C) cm^{-1}, mass spectrum, *m/e*; 254 (M$^+$, 100%), 239 (M$^+$—CH_3, 9), 226 (M$^+$—CO, 13), 225 (M$^+$—CHO, 12), 197 (M$^+$—2CO—H, 26), 77 (25), 76 (29). I diacetate (with Ac_2O/pyridine): cream yellow cubic crystals (from MeOH), m.p. 217–219°, $C_{19}H_{14}O_6$ (Calc. for 338·079) 338·080, IR ν_{max} ($CHCl_3$) 1765 (ester), 1672 (C=O), 1592 (C=C) cm^{-1}, NMR ($CDCl_3$) 2·33 (s, 3H, Ar-CH_3), 2·48 (s, 3H, $OCOCH_3$), 2·52 (s, 3H, $OCOCH_3$), 7·27 (s, 1H, 2-H), 7·76 (m, 2H, 6,7-H), 8·17 (m, 2H, 5,8-H) ppm.

Pachybasin (II). A small amount of yellow pigment Q_2 was obtained from fraction 10–11 (the acidic fraction) and a larger amount from fraction 8–10 (the neutral fraction). Q_2 was acetylated by usual method and recrystallized from MeOH to give light yellow needles (II acetate), m.p. and mixed m.p. 150–152°, $C_{17}H_{12}O_4$ (Calc. for 280·074) 280·071, IR ν_{max} ($CHCl_3$) 1763 (ester), 1670 (C=O), 1607, 1591 (C=C). II acetate was deacetylated to give yellow needles (II, 2·2 mg, from MeOH), m.p. 176–177° and mixed m.p. 175–176°, $C_{15}H_{10}O_3$ (Calc. for 238·063) 238·064, UV λ_{max} (EtOH) 227 (log ϵ 4·28), 240 (sh) (4·36), 246 (sh) (4·42), 255 (4·44), 260 (4·44), 274 (sh) (4·22), 283 (4·16), 336 (3·63), 389 (sh) (3·72), 404 (3·79), 422 (3·72) nm, IR ν_{max} ($CHCl_3$) 1670 (free C=O), 1635 (chelated C=O), 1593 (C=C) cm^{-1}, mass spectrum, *m/e*; 238 (M$^+$, 100%), 223 (M$^+$—CH_3, 17), 210 (M$^+$—CO, 32); 182 (M$^+$—2CO, 54), 165 (M$^+$—2CO—OH, 10), 77 (28), 76 (37).

3-*Methylpurpurin* (III). Brown pigment Q_3 from fraction 12 (the acidic fraction) was acetylated, rechromatographed over a silica gel column and eluted with C_6H_6 to afford trace of dull yellow needles (III triacetate), $C_{21}H_{16}O_8$ (Calc. for 396·085) 396·086. III triacetate was deacetylated to give fine brown-red needles (III, 0·1 mg), $C_{15}H_{10}O_5$ (Calc. for 270·057) 270·053, UV λ_{max} (EtOH) 240 (sh) (log ϵ 4·27), 259 (4·42), 277 (sh) (4·33), 298 (sh) (4·17), 363 (3·56), 384 (3·56), 447 (sh) (3·90), 484 (4·00), 519 (3·89), 571 (sh) (3·35) nm, MS *m/e*; 270 (M$^+$, 100%), 242 (M$^+$—CO, 25), 241 (M$^+$—CHO, 11), 225 (M$^+$—CO—OH, 13), 224 (M$^+$—CHO—OH, 38), 196 (M$^+$—CHO—CO—OH, 18), 77 (23), 76 (16).

3-*Methylalizarin* (IV). Q_4 from fraction 16–20 (the acidic fraction) was acetylated and recrystallized from MeOH to yield light yellow needles (IV diacetate, 0·5 mg), m.p. 212–213°, $C_{19}H_{14}O_6$ (Calc. for 338·079) 338·079. IV diacetate was deacetylated to give orange needles (IV, 0·2 mg), m.p. and mixed m.p. 249–250°, $C_{15}H_{10}O_4$ (Calc. for 254·058) 254·056, UV λ_{max} (EtOH), 249 (log ϵ 4·44), 269 (4·45), 285 (sh) (4·29), 334 (sh) (3·78), 368 (sh) (3·57), 438 (3·71), 580 (sh) (3·04) nm, MS *m/e*; 254 (M$^+$, 100%), 226 (M$^+$—CO, 13), 225 (M$^+$—CHO, 15), 198 (M$^+$—2CO, 6), 197 (M$^+$—2CO—H, 17), 77 (9), 76 (16).

Synthesis of Anthraquinone Pigments

3-*Methylquinizarin* (I). I (0·21 g) was synthesized by condensing phthalic anhydride (1·5 g) with *p*-toluhydroquinone (1·25 g) in fused $AlCl_3$-NaCl according to the modified method of Lovie and Thomson.[9] Crystallization from MeOH gave orange-red needles, m.p. 179° (lit.[10] 160° and lit.[11] 177°) (Found: C, 70·42; H, 3·83. Calc. for $C_{15}H_{10}O_4$: C, 70·87; H, 3·94%). I diacetate was obtained as cream yellow cubic crystals (from MeOH), m.p. 217–219° (lit.[10] 185°) (Found: C, 67·18; H, 4·29. Calc. for $C_{19}H_{14}O_6$: C, 67·46; H, 4·13%).

Pachybasin (II). By the condensation of phthalic anhydride (4·5 g) with *m*-cresol (3·8 g), II (2·1 g) was prepared. Crystallization from MeOH gave yellow needles, m.p. 175–176° (lit.[3] 174·5–175°), (Found: C, 75·52; H, 3·93. Calc. for $C_{15}H_{10}O_3$: C, 75·63; H, 4·20%). II monoacetate; light yellow needles (from MeOH), m.p. 152° (lit.[3] 153°) (Found: C, 72·71; H, 4·27. Calc. for $C_{17}H_{12}O_4$: C, 72·86; H, 4·29%).

1-*Hydroxy-2-methylanthraquinone*. It (0·15 g) was obtained by the condensation of phthalic anhydride (1·5 g) with *o*-cresol (1·3 g). Crystallization from MeOH gave yellow needles, m.p. 184–186° (lit.[12] 184–185°) (Found: C, 75·37; H, 4·21. Calc. for $C_{15}H_{10}O_3$: C, 75·63; H, 4·20%). 1-Acetyl-2-methylanthraquinone: cream yellow needles (from MeOH), m.p. 179–180° (lit.[12] 177–178°) (Found: C, 72·74; H, 4·44. Calc. for $C_{17}H_{12}O_4$: C, 72·86; H, 4·29%).

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[9] J. C. Lovie and R. H. Thomson, *J. Chem. Soc.* 4139 (1959).

[10] R. Nietzki, *Chem. Ber.* **10**, 2012 (1877).

[11] F. Ullmann and W. Schmidt, *Chem. Ber.* **52**, 2110 (1920).

[12] S. Keimatsu and T. Hirano, *J. Pharm. Soc. Japan* **49**, 17 (1929).

3-*Methylpurpurin* (III). III (0·3 g) was prepared from 4-bromorubiadin (1 g) by heating with anhydrous H_3BO_3 (4 g) and conc. H_2SO_4 (15 ml) according to the method of Hirose[13] and recrystallized from MeOH to give brown-red needles, m.p. 265–266° (lit.[13] 266–267°), $C_{15}H_{10}O_5$ (Calc. for 270·057) 270·056. III triacetate; dull yellow needles (from MeOH), m.p. 211–213° (lit. [13] 213°), $C_{21}H_{16}O_8$ (Calc. for 396·085) 396·088.

3-*Methylalizarin* (IV). By the condensation of phthalic anhydride (4·5 g) with 3-methylcatechol (3·8 g), IV (1·5 g) was obtained. Crystallization from MeOH gave orange needles, m.p. 249–251° (lit.[7] 250–251°) (Found: C, 70·67; H, 3·99. Calc. for $C_{15}H_{10}O_4$: C, 70·87; H, 3·94%). IV diacetate; light yellow needles, m.p. 213–214° (lit.[7] 213–214°) (Found: C, 67·75; H, 4·18. Calc. for $C_{19}H_{14}O_6$: C, 67·46; H, 4·13%).

Identification of Anthraquinone Pigments in Intact Plant

A 2-yr-old cultivated intact plant was divided into the aerial part (60·2 g) and root (4·4 g). They were dried, cut finely and extracted with C_6H_6. The C_6H_6 solution, evaporated to a small volume *in vacuo*, was subjected to preparative TLC of silica gel G treated with 0·5 N oxalic acid and developed with C_6H_6–Et_2O (4:1). Two bands corresponding to anthraquinones with α-OH and β-OH groups, respectively, were scraped off and extracted with Me_2CO to give anthraquinone pigments, which were identified as I and II (from α-OH band) and VI (from β-band) by GLC.

TLC and GLC of Anthraquinone Pigments

The experimental results are summarized in the following Table 1.

TABLE 1. TLC AND GLC OF ANTHRAQUINONE PIGMENTS IN CALLUS TISSUE OF *D. lanata*

	TLC					GLC‡
	R_f*					
Compound	A	B	Color on plate (B)	Color under UV	Color with 2N NaOH	t_R (min)
I (Q_1)	0·70	0·70	orange	yellow	purple	2·6
II (Q_2)	0·75	0·65	yellow	red	red	2·0
III (Q_3)	0·05†	0·55	orange	dull yellow	pink	—§
IV (Q_4)	0·15†	0·55	yellow	brown	violet	3·0
V (Q_5)	0·40	0·45	yellow	pale yellow	red	3·9
VI (Q_6)	0·45	0·40	pale yellow	greenish yellow	red	3·3

* A, silica gel G, solvent: C_6H_6–Et_2O (4:1). B, silica gel G treated with 0·5 N oxalic acid, same solvent as A.

† Tailing.

‡ 1% SE-30 on Gas-Chrom Q (80–100 mesh), Column length 1·8 ml, Column temp. 215°, Detector temp. 250°, Carrier gas N_2, flow rate 60 ml/min.

§ Not recorded.

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[13] Y. HIROSE, *Chem. Pharm. Bull. Tokyo* **8**, 417 (1960).

Key Word Index—Digitalis lanata; Schrophulariaceae; anthraquinones; 3-methylpurpurin; 3-methylquinizarin; pachybasin; 3-methylalizarin.