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ANTHRAQUINONES IN *DIGITALIS* SPECIES

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Abstract—Twenty anthraquinones have been found in the roots of *Digitalis trojana* of which the following are new: digitopurpone-1-methyl ether, ω-hydroxypachybasin and ω-hydroxyziganein. The biogenesis of the thirty anthraquinones now known in *Digitalis* s.l. is discussed.

Digitolutein, the first anthraquinone found in *Digitalis*, was isolated in 1899 [1], and again in 1954 [2], but the structure **10** was not determined until 1955 [3]. Anthraquinones remained as rare pigments in *Digitalis* until 1968 [4] since when numerous quinones have been described [5–14]. We report here on three new anthraquinones in *D. trojana* Ivan.

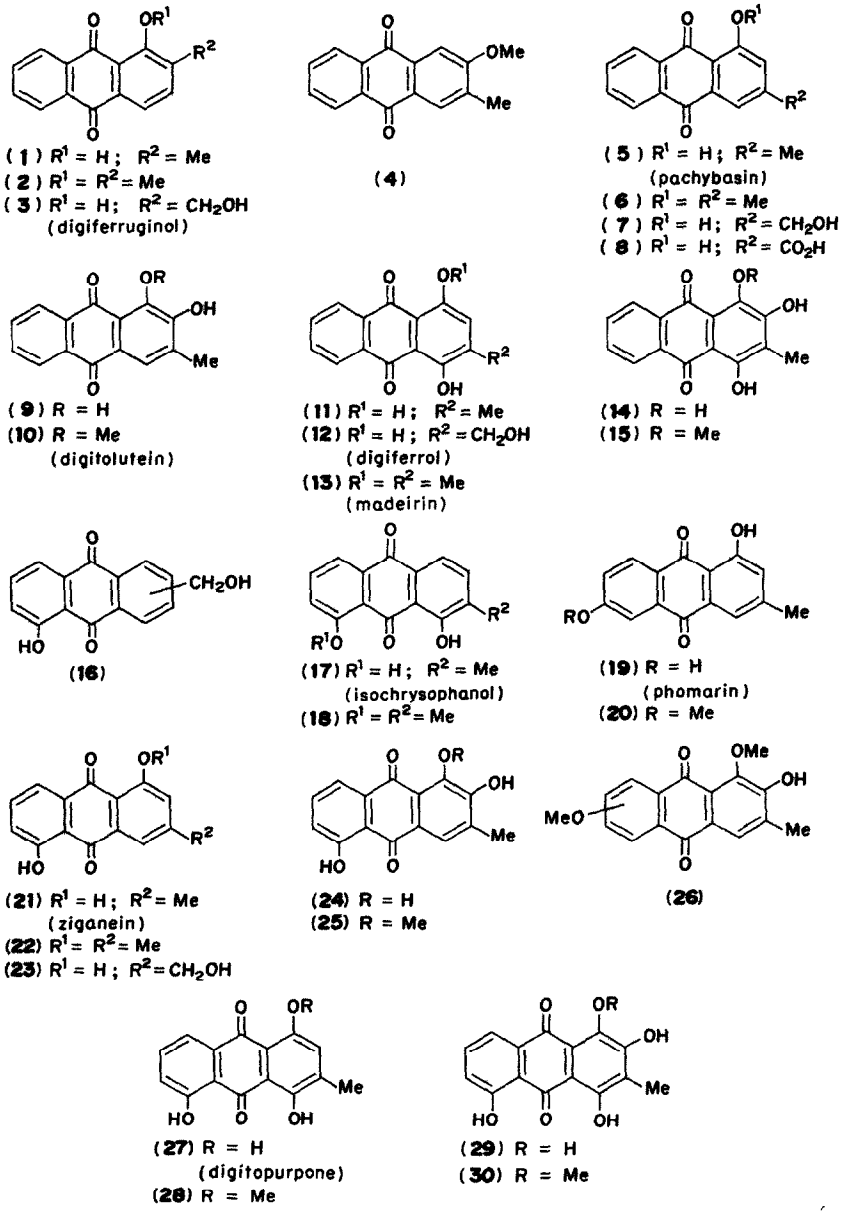
By extraction of the roots of *D. trojana* with 96% ethanol followed by extensive column chromatography and PLC twenty anthraquinones were separated (see Table 1) but in some cases the amount was too small to permit isolation. The known compounds 1-hydroxy-2-methylanthraquinone **1**, pachybasin **5**, 1-hydroxy-6(or 7)-hydroxymethylanthraquinone **16**, digitolutein **10**, madeirin **13**, 4-hydroxydigitolutein **15**, phomarin **19**, ziganein **21** and its monomethyl ether **22**, 5-hydroxydigitolutein **25**, and 4,5-dihydroxydigitolutein **30** were identified by direct comparison (mp, TLC, UV, IR) with authentic samples, while 3-methylalizarin (nor-digitolutein) **9**, 2-methylquinizarin **11**, 3-methylpurpurin **14**, and digitopurpone **27** were identified by comparative TLC (in 3 systems) only. The compounds 5-hydroxy- **24**, and 4,5-dihydroxy-nor-digitolutein **29** were not isolated but were also identified by comparative TLC (in 3 systems) with authentic specimens obtained by demethylation of the corresponding hydroxydigitoluteins. Three new natural pigments (A), (B), (C) were isolated.

From the PMR spectrum pigment A contains a Me, a OMe, and two *peri*-OH groups, an isolated aromatic proton (δ 7·14) and three other aromatic protons. The IR spectrum shows free and chelated carbonyl absorption at 1660 and 1624 cm^{-1} but after demethylation only one carbonyl peak is observed at 1606 cm^{-1}. Thus the OMe group occupies an α-position, and as the pigment gave digitopurpone **27** on demethylation it must be digitopurpone-1-methyl ether **28**.

Table 1. Distribution of anthraquinones in *Digitalis* s.l.

	Anthraquinones*																													
Digitalis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17**	18	19	20	21	22	23	24	25	26	27	28	29	30	Ref.
Section Digitalis																															
D. purpurea L		+		+		+			+	+					+		+		+	+							+				2,4,20
Section Macranthae																															
D. grandiflora Miller																			+												9
Section Tubiflorae																															
D. lutea L.										+																					1
D. viridiflora Lindley										+									+												5,6,9
D. viridiflora† Lindley																		+							+						11
Section Globiflorae																															
D. cariensis Boiss. ex Jaub. and Spach																															
ssp. *lamarkii* (Ivan.) Werner‖				+			+		+			+			+	+			+		+	+			+						13
ssp. *trojana* (Ivan.) Werner	+			+		+		+	+	+	+		+	+	+	+			+		+	+	+	+	+			+	+	+	+ h
D ferruginea L.			+									+							+												8a
ssp. *schischkinii* (Ivan.) Werner	+			+		+		+	+	+	+		+	+	+	+	+		+		+	+	+	+	+			+	+	+	+ 14
D. lanata Ehrh		+		+						+																					4
D. lanata‡ Ehrh					+				+	+	+			+	+																25
Isoplexis																															
I. canariensis (L.) Lindley ex G. Don¶				+		+																									20, 20a
I. sceptrum (L.f.) Lindley ex G. Don§				+	+						+		+																		10

* In leaves unless otherwise stated. † In roots. ‡ In seeds (TLC). ‖ *D. orientalis* Lam. ¶ *D. canariensis* L. § *D. sceptrum* Linn. ** This structure has been assigned incorrectly to a quinone, mp 307–309°, found in the seeds of *Cassia occidentalis* [27]. The *Cassia* pigment is probably a bianthraquinone. †† Present work.



Pigment **B**, $C_{15}H_{10}O_4$, absorbed at λ_{max} 253 nm and ν_{max} 3500, 1656 and 1633 cm^{-1}, and the PMR spectrum (in DMSO-d_6) included a 2H doublet at δ 4·64 and an OH triplet [15] at δ 5·58, O-Me and C-Me signals being absent. This evidence is consistent with a 1-hydroxy-X-hydroxymethylanthraquinone structure, and direct comparison showed that the pigment was identical with the 1,3-isomer **7** previously synthesised [8b].

Pigment **C**, $C_{15}H_{10}O_5$, showed λ_{max} 418 and 428 nm, and γ_{CO} 1616 cm^{-1} indicative of a 1,5-dihydroxyanthraquinone structure. It also contains a hydroxymethyl group which is responsible for a 2H doublet at δ 4·63 in the PMR spectrum (in DMSO-d_6), and an intense M^+-29 peak in the MS. The latter, which is frequently the base peak (as in this case) is normal in benzyl alcohols [16] and we find it of diagnostic value for AQ-CH_2OH. Pigment **C** is therefore most probably 1,5-dihydroxy-2 or 3-hydroxymethylanthraquinone and it was identified as the 3-isomer **23** by direct comparison with a synthetic sample [17].

Thirty anthraquinones have now been identified in *Digitalis* (see Table 1) and others have been detected. They generally occur in leaves, but have also been found in roots (see Table 1) and there is some evidence for their presence in seeds [18]. So far they have not been found in other genera of the Scrophulariaceae [19] apart from two species of *Isoplexis* formerly assigned to *Digitalis*. It may be significant that digitolutein **10** (R = Me), the most characteristic *Digitalis* quinone, was not found in these two species. However, the work on *I. canariensis* was not concerned with pigments, and the presence of other anthraquinones in small amount could easily have been overlooked. The distribution pattern suggested by Table 1 is probably misleading. A more systematic reinvestigation of some of the earlier work would almost certainly reveal the presence of other quinones *D. gran-*

diflora and *D. lutea* are obvious candidates for further study.

A few of these pigments also appear in other major groups. Thus quinone **1** [20] occurs in Rubiaceae and Bignoniaceae, and **5**, [20] **11**, [20] and **25** [21] in Verbenaceae, and the quinones in these three families show a general resemblance to those of *Digitalis*. The main feature is the predominant substitution in ring C but only one substituent (OH or OMe), or none, in ring A. Biosynthesis by the shikimate—*o*-succinoylbenzoic acid pathway seems likely which has been established for Rubiaceae [22] and also for Gesneriaceae (quinone **3**) [23]. However, pachybasin **5** and phomarin **19** are also fungal products, and indeed they co-occur with chrysophanol and emodin in cultures of *Phoma foveata* [24]. These are products of polyketide origin which suggests the possibility that two pathways may be utilised by *Digitalis*. These problems can only be solved by feeding experiments, a major difficulty being the exceedingly small quantity of quinones normally present in *Digitalis* leaves. Tissue culture may provide the answer as it is known that callus tissue of *D. lanata* contains several anthraquinones (**5**, **9**–**11**, **14**, **15**) [25].

EXPERIMENTAL

Spectra were run in MeOH (UV), KBr (IR), and $CDCl_3$ (PMR) unless otherwise stated. Solvent systems for TLC on polyamide were (A) MeOH and (B) MeOH–MeCOEt (15:1), and for TLC and PLC on Si gel (C) hexane–Et_2O (1:1) and (D) petrol (bp 50–70°)–EtOAc (15:1). All quinones were crystallised from MeOH, and were identified by direct comparison (TLC, UV, IR) with authentic samples, except quinone **28**.

Extraction. D. trojana was collected in July 1972 on Kazdagi in Turkey. Coarsely powdered roots (4·8 kg) were exhaustively percolated with 96% EtOH. After removal of solvent, H_2O (1 l.) was added, followed by extraction with C_6H_6. Removal of the C_6H_6 left a residue (37·5 g) which was transferred to a column (4·8 × 60 cm) of acidic Si gel (SiO_2 shaken with 5% $(CO_2H)_2$ for 30 min, filtered and dried at 90° for 2 hr.) and eluted with C_6H_6; 2995 20 ml fractions were collected. These were combined into 8 major quinone-containing fractions and processed as follows. Fractions 1–50 were defatted by treatment with *N* NaOH, followed by acidification, re-extraction into C_6H_6 and evaporation. Residue was chromatographed on a polyamide column (1·8 × 28 cm) in MeOH–H_2O (1:1) to give ziganein **21**, orange needles, mp 222–224° (lit. [12] 224–227°) (3·5 mg), digitopurpone **27**, and 2-methylquinizarin **11**. The residue from fractions 51–79 when purified by PLC in solvent (D) yielded pachybasin **5**, dark yellow needles, mp 173° (lit. [26] 175°) (11·5 mg). The trace quinones in fractions 80–754 could not be isolated but were identified (TLC in A, B, and D) as 3-methylpurpurin **14**, and 3-methylalizarin **9**, 5-hydroxy-**24** and 4,5-dihydroxynordigitolutein **29**. Fractions 1146–1505 were rechromatographed on a column (2 × 44 cm) of Si gel in CCl_4, collecting 275 25 ml fractions. Fractions 121–157 yielded, after PLC in solvent (C), 4-hydroxydigitolutein **15**, orange needles, mp 222–224° (lit. [7] 220°) (2·4 mg). Fractions 158–275 were rechromatographed on a polyamide column (1·8 × 25 cm) in MeOH–H_2O (1:1) to give 5-hydroxydigitolutein **25**, orange needles, mp 244–245° (lit. [11, 21] 234–236°, 237°) (20·5 mg) (Found: M^+ 284·0685. $C_{16}H_{12}O_5$ requires M, 284·0684) λ_{max} 254*sh*, 272 *sh*, 280, 412 nm (log ε 4·21, 4·32, 4·34, 3·85), ν_{max} 1666, 1630, 1588 cm^{-1}, and 4,5-dihydroxydigitolutein **30**, orange–red needles, mp 239–242° (lit. [14] 244°) (1·8 mg). Fractions 1540–1930 were further separated by PLC in solvent (C), to give ziganein-1-methyl ether **22**, dark yellow needles, mp 198–200° (lit. [12] 197–199°) (75 mg) and a mixture of **10** and **25**. The latter was passed down a short polyamide column in MeOH–H_2O to give digitolutein **10**, yellow needles, mp 222–224° (lit. [3] 222°) (20·7 mg). Fractions 1931–2206 yielded, after PLC in solvent (C), madeirin **13**, red needles, mp 186–188° (lit. [10] 188–190°) (13 mg). Similarly fractions 2207–2336 afforded digitopurpone-1-methyl ether **28**, orange needles, mp 207–209° (9·7 mg) (Found: M^+, 284·0688. $C_{16}H_{12}O_5$ requires M, 284·0684) λ_{max} 231, 249 *sh*, 284 *sh*, 472 nm (log ε 4·45, 4·07, 3·82, 3·87), ν_{max} 1660, 1624, 1594 cm^{-1}, δ 2·36 (3 H, *s*, Me), 3·97 (3 H, *s*, OMe), 7·14 (1 H, *s*, ArH), 7·53–7·85 (3 H, *m*, ArH), 11·93 and 12·68 (each 1 H, *s*, OH), *m/e* (%) 284(100), 267(13), 266(4), 256(11), 255(71), 241(18), 239(7), 227(6), 213(9), 181(5), 158(5·5), 152(7), 139(15). [Quinone **28** (3·5 mg) was demethylated with $AlCl_3$ in hot C_6H_6 (4 ml) to give digitopurpone, red needles, mp 208–209° (lit. [7] 209–211°) (1·8 mg)]; phomarin **19**, orange needles, mp 264–266° (lit. [7] 259·5–260·5°) (15 mg), and 1-hydroxy-2-methylanthraquinone **1**, yellow needles, mp 175–177° (lit. [8b] 180–182°) (1·4 mg). Fractions 2374–2697 were partially purified by PLC on Si gel/5% $(CO_2H)_2$ in solvent (C) and then passed down a short polyamide column in MeOH–H_2O to yield *ω-hydroxyziganein* **23**, orange crystals, mp 232–234° (lit. [17] 233–234°) (12 mg) (Found: M^+, 270·0529. $C_{15}H_{10}O_5$ requires 270·0528), λ_{max} 254, 279 *sh*, 289, 418, 428 nm (log ε 4·37, 4·03, 4·04, 4·06, 4·06), ν_{max} 1616 cm^{-1}, δ (DMSO-d_6) 4·63 (2 H, *d*, CH_2OH), 5·61 (1 H, *m*, $CH_2\underline{OH}$), 7·20–7·86 (5 H, *m*, ArH), 12·36 (2 H, *s*, OH), *m/e* (%) 270(70), 241(100), 224(9), 213(13), 196(8), 185(5), 168(10), 155(7), 139(22). By repeated PLC on SiO_2/5% $(CO_2H)_2$ in solvent (C) fractions 2698–2945 afforded two quinones: 1-hydroxy-6 or 7-hydroxymethylanthraquinone **16**, yellow needles, mp 208–210° (lit. [13] 203–206°) (9 mg), ν_{max} 3420, 1672, 1641, 1605 cm^{-1}, *m/e* 254 (M^+, 100%), 225 (M^+–29, 56%), and ω-hydroxypachybasin **7**, yellow needles, mp 210–212° (lit [8b] 210°) (16 mg), ν_{max} 3500, 1656, 1633, 1590 cm^{-1}, δ (DMSO-d_6) 4·64 (2 H, *d*, CH_2OH), 5·58 (1 H, *t*, $CH_2\underline{OH}$), 7·35 (1 H, *bs*, ArH), 7·76 (1 H, *bs*, ArH), 7·94 (2 H, *m*, ArH), 8·20 (2 H, *m*, ArH), 12·09 (1 H, *s*, *peri* OH), *m/e* 254 (M^+, 100%), 225 (M^+-29, 79%).

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