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4-HYDROXYDIGITOLUTEIN, A NEW ANTHRAQUINONE FROM CALLUS TISSUE OF DIGITALIS *LANATA**

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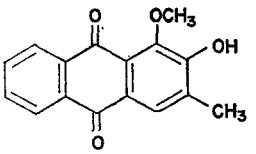
Abstract—Many anthraquinone derivatives were isolated from the callus tissue of *Digitalis lanata* (Scrophulariaceae). Two of them were digitolutein (I) and a new anthraquinone 4-hydroxydigitolutein (II). The chemical structure of 4-hydroxydigitolutein was established to he 3-methylpurpurin l-methyl ether by mass i.r. and NMR spectra and mixed melting point test with synthetic sample. Anthraquinone derivatives have been found for the first time in plant tissue cultures.

INTRODUCTION

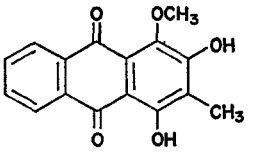
VERY little is known about the chemical constituents of digitalis callus tissues.[1,2] During the course of the investigation on the digitalis cardiotonic substances in the callus tissue of ***Digitalis lanata*** in our laboratory, yellow spots which gave red and orange colors with Kedde reagent[3] were observed. Büchner and Staba[1] also reported the yellow spots which gave a red color with the same reagent in chloroform extracts of the callus tissue of ***Digitalis Zanata.*** We assumed them to be the pigments belonging to anthraquinone derivatives and carried out their isolation and elucidation of their chemical structures.

RESULTS

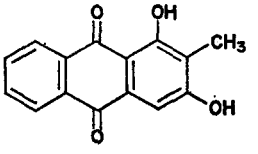
The pigment fractions extracted and separated from the callus tissue were put on silica gel columns treated with 0·5 N oxalic acid. Six pigments designated as Q_1, Q_2, Q_3, Q_4, Q_5 and Q_6 were isolated, but the first four were isolated only in trace amounts, Q_6 was identified as digitolutein (I) in comparison with authentic sample by means of TLC, GLC and mass spectral analyses. The pigment Q_5 (II) was the main component. I gave the molecular formula $C_{16}H_{12}O_5$ with its diacetate $C_{20}H_{16}O_7$ by high resolution mass



digitolutein (I)



4—hydroxydigitolutein (II)



rubladln (III)

spectrometric measurement. Its mass spectrum indicated that the molecular peak at m/e 284 (base peak) was 16 mass weight larger than that of digitolutein and its pattern was very similar to that of digitolutein but was different from that of isomer, obtusifolin.[4] The u.v. spectrum of Q_5 was similar to that of digitolutein and alizarin l-methyl ether. The i.r.

* Part IX in the series "Studies in Plant Tissue Cultures"; for Part VIII, see T. FURUYA, M. HIROTANI and T. SHINOHARA, Chem. *Pharm. Bull.* 18, 1080 (1970).

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spectrum showed the presence of a chelated carbonyl (1618 cm^{-1}) and a non-chelated carbonyl(1667 cm-'). From these data and the solubility in 5 % Na_2CO_3, Q_5 presumed to be the anthraquinone derivative having a chelated and a non-chelated hydroxyl groups. As shown in Table 1, NMR spectrum of Q_5 assumed the presence of two methyl groups 2·27 ppm (Ar-CH,) and 3·98 ppm (OCH,). The four protons at 7·76 ppm (multiplet. 2H)

TABLE 1. MAIN SIGNALS OF NMR SPECTRA OF 4-HYDROXYDIGITOLUTEIN (II) AND RELATED COMPOUNDS*

Compounds	Ar-CH_3	β-OAc	a-OAc	OCH_3	3-H	6.7-H	5,8-H	4-H
Alizarin diacetate		2.38	2·52		7.42 (d, J = 10)	7.77 (m,2H)	8.18 (m,2H)	8.22 (d)
(II) Diacetate	2·14	2.40	2.50	3.94		7.76 (m,2H)	8.20 (m,2H)	
(11)	2.27			3.98		7.76 (m,2H)	8.25 (m,2H)	

* The spectra were determined in $CDCl_3$ containing TMS as an internal reference at 100 Mc/sec except alizarin diacetate at 60 Mc/sec. Unless otherwise indicated, all signals are singlets. In other case d = doublet, m = multiplet and J are given in c/s. Chemical shifts are on the δ scale.

and 8.25 ppm (multiplet, 2H) of Q_5 are due to aromatic protons of one benzenoid ring, as indicated in alzarin diacetate. The signals of 3-H and 4-H in alizarin diacetate were not observed in Q_5. Therefore, Q_5 seemed to be alizarin type having no functional groups in one benzenoid ring. Acetyl methyl protons of Q_5-diacetate at 2·40 ppm and 2·50 ppm presented β- and a-positions, respectively, and methyl protons (Ar-CH,) shifted little to higher field in comparison with that of Q_5 due to the effect of two acetyl groups in *ortho* position.'

From the results above, it was concluded that the chemical structure of Q_5 is 3-methylpurpurin l-methyl ether (II). So Q_5 is a new naturally occurring anthraquinone and named as 4-hydroxydigitolutein. The structure was confirmed by synthesis from rubiadin (III). (III) was converted into 4-bromorubiadin diacetate via 4-bromorubiadin. Methoxylation of 4-bromorubiadin diacetate with sodium methoxide in anhydrous benzene and successional hydrolysis gave 3-methylpurpurin l-methyl ether (II), which was proved to be identical with natural 4-hydroxydigitolutein by mixed melting point, i.r., NMR and mass spectral analyses.

Further, the confirmation was made by demethylation of synthetic and natural (II) with borontribromide to afford 3-methylpurpurin, which was identical with that obtained by hydroxylation of 4-bromorubiadin.

DISCUSSION

Although digitolutein have been already isolated from the leaves of *D. lutea*,[6] *D. purpurea*,[7,8] *D. lanata*[9] and *D. viridiflora*,[10] it together with 4-hydroxydigitolutein has been found for the first time in the callus tissue of *D. lanata*. Therefore, the presence of anthraquinone derivatives has been demonstrated for the first time in plant tissue cultures. 4-Hydroxydigitolutein and digitolutein have the substituents in one benzenoid ring only and

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the same structural type as that contained in Verbenaceae, Bignoniaceae and Rubiaceae. Some anthraquinones such as 1-methoxy-3-methylanthraquinone," 1-methoxy-2-methylanthraquinone,[9] 3-methoxy-2-methylanthraquinone,[9] 3-methylalizarin,[9] 1,6 (or 1,7)-dihydroxy-3-methylanthraquinone[10] and 2-hydroxy-1,6 (or 1,7)-dimethoxy-3-methylanthraquinone[12] are found in the leaves of *Digitalis* species until now and belong to this type except the latter two compounds. So the biosynthesis of anthraquinones in digitalis callus tissue is of much interest in comparison with that in Rubiaceae[13,14] and Polygonaceae plants.[15]

EXPERIMENTAL

M.ps were determined in a Büchi apparatus and uncorrected. The NMR spectra were run in a JNN-4H-100 and a Hitachi H-60NMR spectrometer. The mass spectra were taken on a JMS-OIS double-focusing mass spectrometer with direct inlet system.

Tissue Culture of D. lanata

Seed of *D. lanata* was aseptically germinated and seedling were transferred onto White's basal medium containing 2.4-dichloroohenoxyacetic acid 1 ppm, yeast extract (Difco) 0·1% and sucrose 2% in February 1965. The callus tissue-was subcultured every 3 weeks. A part 'of this callus tissue was transferred onto Murashige and Skoog's basal medium containing 3-indolylacetic acid 1 ppm, kinetin 0·1 ppm and sucrose 3% in July 1967. This callus tissue has been subcultured.

Isolation of Anthraquinone Pigments from Callus Tissue

The callus tissue 224 g was homogenized with cold acetone 300 ml, refluxed for 3 hr and filtered. This process was repeated 3 times, and the filtrate evaporated. The concentrated aqueous solution was extracted with benzene, and the benzene solution was shaken with 5% $NaHCO_3$, 5% Na_2CO_3 and 5% NaOH. Each basic solution after acidification was re-extracted with benzene, and the separate benzene solutions after drying (Na_2SO_4) evaporated to dryness. The fractions obtained were designated A, B, C respectively and that from the original benzene D.

The mixed fraction of C and D was eluted with benzene using a deactivated silica gel column* (12 g) to give two main bands (each fraction; 10 ml). The first gave a crystalline compound Q_1, which was recrystallized from MeOH to give red needles and the second gave yellow needles Q_2, after the same treatment: both compounds were found in very small traces.

Fraction B (82.6 mg) on deactivated silica gel column* (40 g) and eluted with benzene (each fraction; 100 ml) gave traces of red and brown pigments, Q_3 and Q_4 respectively. Fractions 15-19 (5·2 mg) containing Q_5 was acetylated by usual method and rechromatographed to give the pure diacetate of Q_5 (5·0 mg). The pigment Q_6 (2.1 mg) was obtained from fraction Nos. 30–32 as yellow needles, m.p. 218-219". Fraction Nos. 35-37 gave trace of yellow pigment.

Properties of 4-Hydroxydigitolutein (Q_5)

4-Hydroxydigitolutein. Orange needles (from MeOH), m.p. 217·5–218°, $C_{16}H_{12}O_5$ (requires 284068) 284.063, u.v. λ_{max} (EtOH) 244 mμ (sh) (log ε 4·39), 250 mμ (4·42), 275 mμ (4·31), 296 mμ (sh) (4·09), 413 mμ (sh) (3·74), 438 mμ (3·80), 460 mμ (sh) (3·72), i.r. ν_{max} (KBr), 3360 (OH), 1667 (non-chelated C=O), 1618 (chelated C===O), 1593 (double bond) cm^{-1}, mass spectrum, *m/e;* 284 (M^+, 100%), 269 ($M^+ - CH_3$, 8%) 267 (M+ — OH, 18%), 266 (M+ — H_2O, 80%), 255 (M+ — CHO, 19%), 241 ($M^+ - CH_3$ — CO, 37%), 210 (M^+ -2 x CO — H_2O, 35%), 77 (9%), 76 (8%).

4-Hydroxydigitolutein diacetate. Light yellow needles (from MeOH), m.p. 187–188°, $C_{20}H_{16}O_7$ (requires 368·090) 368.084, i.r. ν_{max} (KBr) 1776, 1270, 1186 (ester), 1678 (C=O) cm^{-1}, mass spectrum, *m/e;* 368 (M^+, 9%), 326 (M+ — $COCH_2$, 68%), 284 (M+ — 2 x $COCH_2$, 100%), other peaks gave similar ones to 4-hydroxydigitolutein.

* Silica gel Kanto Kagaku was stirred with 0·5 N oxalic acid, filtered and dried at 100" for 1 hr.

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Synthesis of 4-Hydroxydigitolutein

Rubiadin (III) was synthesized by the condensation of phthalic anhydride with 2-methylresorcinol in fused $AlCl_3$–NaCl according to the modified method of Lovie and Thomson.[16] Crystallization from MeOH gave yellow needles, m.p. 287-288" (lit.[17] m.p. 290°). (Found: C, 71·04; H, 3.99. Calc. for $C_{15}H_{10}O_4$: C, 70.87; H, 3·94%.) 4-Bromorubiadin was obtained by bromination of (III) with Br_2 in glacial HOAc containing NaOAc.[18] Crystallization from MeOH gave yellow needles, m.p. 206–208° (lit.[18] m.p. 203-205"). (Found: C, 53.49; H, 2·71. Calc. for $C_{15}H_9O_4Br$: C, 54·05; H, 2·70%.) 4-Bromorubiadin diacetate was prepared from Cbromorubiadin in usual manner with pyridine and Ac_2O to give the light yellow needles, m.p. 214–216°. (Found: C, 5453; H, 3.13. Calc. for $C_{19}H_{13}O_6Br$: C, 54.68; H, 3·12%.) To NaOMe (freshly prepared) an anhydrous benzene solution of 4-bromorubiadin diacetate (100 mg) was added and refluxed for 24 hr. The reaction mixture was poured into 2N NaOH solution, boiled for 10 min, acidified with 2N HCl and collected. As the product contained a few subproducts, it was purified by the column chromatography. Crystallization from glacial HOAc gave orange needles, 14.5 mg (yield 21·3%), m.p. 217–218°, $C_{16}H_{12}O_5$ (requires 284.068) 284·069. This compound was identified with natural 4-hydroxydigitolutein by mixed m.p., i.r., NMR and mass spectral analyses.

Demethylation of 4-Hydroxydigitolutein

4-Hydroxydigitolutein (II) 2 mg was dissolved with small BBr_3 and stood overnight. The extract with benzene from residue was proved by TLC to be identical with 3-methylpurpurin prepared by the hydroxylation of 4-bromorubiadin according to the method of Hirose.[17]

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