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ANTHRAQUINONES FROM THE ROOTS OF *DIGITALIS GRANDIFLORA*

DIGITALIS GRANDIFLORA KÖK ANTRAKİNONLARI

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The anthraquinones in the roots of Digitalis grandiflora were examined and fourteen known and five unknown anthraquinone derivatives were isolated. The known compounds were identified by comparison of their chromatographic and spectral data with those of authentic material. Structures of four unknown anthraquinones could not be established because of their insufficient amounts. The structure of one of them was determined as 4-hydroxy- 1,2-dimethoxy-3-methylanthraquinone (4-hydroxydigitolutein-2-methylether).

Digitalis grandiflora kökünün antrakinonları incelendi ve 14 bilinen ve 5 bilinmeyen antrakinon bileşiği izole edildi. Bilinen antrakinon bileşikleri test maddelerle kromatografik ve spektroskopik yönden karşılaştırılarak teşhis edildi. Bilinmeyen bileşiklerin miktarları çok az olduğundan 4 tanesinin yapısı aydınlatılamadı. Birisinin yapısı ise spektroskopik yöntemler yardımıyla 4-hidroksi-1,2-dimetoksi-3-metilantrakinon (4-hidroksidigitolutein-2-metileter) olarak aydınlatıldı.

Keywords: *4-hydroxy-1,2-dimethoxy-3-methyl-anthraquinone; Digitalis grandiflora; Anthraquinone*

Anahtar Kelimeler: *4-Hidroksi-1,2-dimetoksi-3-metilantrakinon; Digitalis grandiflora; Antrakinon*

Introduction

It is well known that genus *Digitalis* is qualitatively rich in anthraquinone pigments. However most of these pigments occur in the plant in very small amounts. We have isolated several new pigments and have elucidated their structures in the course of our investigations on anthraquinones of *Digitalis* species growing wild in Turkey (1-4). In the present paper we examined the roots of *Digitalis grandiflora* which was an uninvestigated species.

Material and methods

General procedure: Melting points were determined on a melting point microscope (Reichert) and were uncorrected. The UV and IR spectra were recorded by a Shimadzu 160 A spectrophotometer in MeOH and Perkin-Elmer 1600 FTIR in KBr respectively. The NMR and EIMS were taken with 200 MHz Bruker in $CDC1_3$ and VG 2AB respectively. Kieselgel 60 (0.063-0.200 Merck) and Ultramid B 12 (60-150 nm) were used for column chromatography. For the preparation of acidic adsorbent, silicagel was shaken with 5% $(C0_2H)_2$ for 30 min, filtered and dried at 90^0 for 2 hr. Kieselgel 60 and Polyamide 11 F_{254} (Merck) precoated plates were used for TLC. Solvent systems: for polyamide; MeOH, for silicagel; petroleum ether (40-60^0)/EtOAc/AcOH (74:24:1) and n-Hexane/$CHC1_3$/MeOH (6:3: 1).

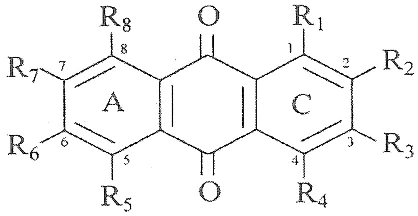
Plant material: Digitalis grandiflora plants were collected from Demirköy/Kırklareli and identified at The Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Istanbul, where a voucher specimen is deposited (ISTE 14570).

Extraction and isolation: The coarsely powdered roots (550 g) were exhaustively percolated with 96% EtOH. After the removal of EtOH, the residue was diluted with H_2O (1/1) and extracted priorly with n-hexane and than with Et_2O and the solvents were removed in vacuum. The residues of both extracts were chromatographed on acidic silicagel columns with petroleum ether (50^0-75^0) and increasing amounts of $CHCl_3$. The compounds **1-15** and **16-20** were isolated and enriched by repeated column chromatography and/or preparative thin layer chromatography from fractions of hexane and Et_2O-extract containing the separated anthraquinones in groups respectively.

Results and Discussion

The ethanolic root extract of *Digitalis grandiflora* was fractioned on a silicagel column and 19 anthraquinone pigments were determined by TLC (**1-19**) Schme. Ten of them were isolated in pure state by column chromatography and preparative thin layer chromatography. Nine pigments were identified by comparison with authentic samples (mp, TLC, UV, IR) as follows: **1**: Digitopurpone **2**: Ziganein **3**: Pachybasin **7**: lsochrysophanol-8-methyl-ether **8**: Ziganein-l-methylether **9**: Digitopurpone-1-methyl ether **14**:ω-hydroxy-pachybasin **15**: ω-hydroxyziganein **17**: ω-hydroxyisochrysophanol-8-methylether. One of these compounds(**5**) showed no similarity with known *Digitalis* anthraquinones at TLC controls.

The remaining anthraquinones in the fractions were in very small amounts and therefore they could be identified only by TLC comparison (in tree systems) with authentic samples as; **10**:Digitolutein **11**:5-Hydroxydigitolutein **12**:6-Methoxydigitolutein **13**:Digiferrol **19**:1-Hydroxyanthraquinone-3-carboxylic acid. By comparative TLC the pigments **4, 6, 16, 18** observed to be were not identical with any known digitalis anthraquinones.



(**1**) $R_1 = R_4 = R_5 = OH$, $R_3 = CH_3$, others = H
(**2**) $R_1 = R_5 = OH$, $R_3 = CH_3$, others = H
(**3**) $R_1 = OH$, $R_3 = CH_3$, others = H
(**5**) $R_1 = R_2 = OCH_3$, $R_3 = CH_3$, $R_4 = OH$, others = H
(**7**) $R_1 = OH$, $R_2 = CH_3$, $R_8 = OCH_3$, others = H
(**8**) $R_1 = OCH_3$, $R_3 = CH_3$, $R_5 = OH$, others = H
(**9**) $R_1 = OCH_3$, $R_3 = CH_3$, $R_4 = R_5 = OH$, others = H
(**10**) $R_1 = OCH_3$, $R_2 = OH$, $R_3 = CH_3$, others = H
(**11**) $R_1 = OCH_3$, $R_2 = R_5 = OH$, $R_3 = CH_3$, others = H
(**12**) $R_1 = R_6 = OCH_3$, $R_2 = OH$, $R_3 = CH_3$, others = H
(**13**) $R_1 = R_4 = OH$, $R_3 = CH_2OH$, others = H
(**14**) $R_1 = OH$, $R_2 = CH_2OH$, others = H
(**15**) $R_1 = R_5 = OH$, $R_3 = CH_2OH$, others = H
(**17**) $R_1 = OH$, $R_2 = CH_2OH$, $R_8 = OCH_3$, others = H
(**19**) $R_1 = OH$, $R_3 = COOH$, others = H
(**20**) $R_1 = OCH_3$, $R_2 = R_4 = OH$, $R_3 = CH_3$, others = H

Scheme- Anthraquinone pigments of Digitalis grandiflora.

Compound **5**, $C_{17}H_{14}O_5$ exhibited a molecular ion peak at m/z 298 as base peak in the EIMS. The IR spectrum had a free (1670 cm^{-1}) and a hydrogen-bonded (1626 cm^{-1}) carbonyl absorption. The ^1H NMR spectrum indicated that Compound **5** contained a *peri*-OH, a C-methyl and two O-methyl groups and four aromatic protons as two multiplets (δ: 7.77 and 8.27) which indicated that ring A is unsubstituted (5). All these evidences suggested that compound **5** must be 2-methylether of 4-hydroxydigitolutein (2,4-dihydroxy-1-methoxy-3-methylanthraquinone, **20**). A comparison of the ^1H NMR data of **5** and **20** (Table 1) supported this suggestion and therefore the number of digitalis anthraquinones rose up to thirtythree.

Table 1. ^{1}H-NMR spectral data of compounds **5** and **20**.

	δ H	
Proton	**5**	**20** [2]
4-OH	13,70 s	
5-H, 8-H	8,27 m	8,25 m
6-H, 7-H	7,77 m	7,76 m
1-OCH$_3$	3,99 s	3,98 s
2-OCH$_3$	3,93	
1-CH$_3$	2,28	2,27 s

5 (4-Hydroxydigitolutein-2-methyl-ether): Yellow needles from MeOH (2.5 mg), mp 112-114^{0}, UV (λ_{max} nm): 206, 249.5, 326, 430.5; IR (ν cm^{-1}) 1670, 1626; ^{1}H-NMR: Table 1. EIMS: *m/z* (rel. int.): 298 M^{+} (100), 283(20), 269(36), 255(20), 167(12), 149(32).

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