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STRUCTURE AND BIOSYNTHESIS OF A NEW ANTHRAQUINONE FROM *STREPTOCARPUS DUNNII*

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Abstract—A new anthraquinone (1-hydroxy-2-hydroxymethylanthraquinone) has been isolated from the title plant. The structure was determined by spectroscopy and synthesis. Investigation on the biosynthesis showed that this quinone is formed via *o*-succinoylbenzoic acid.

INTRODUCTION

DURING an investigation on the biosynthesis of the naphthaquinone dunnione we observed in extracts of leaves and roots of *Streptocarpus dunnii* several unknown yellow compounds. The major product was isolated by chromatography and purified by recrystallisation. Structure and biosynthesis of this compound are presented in this report.

RESULTS AND DISCUSSION

The UV and IR spectra of the main compound showed the absorption typical of 1-hydroxy-anthraquinone: λ_{max}(MeOH) 222, 253, 328 and 407 nm; ν_{max}(KBr) 1672, 1638 cm^{-1}. The NMR spectrum gave signals from 6 aromatic protons, a chelated hydroxy group, and a singlet (2 H) at $\delta = 4{\cdot}69$ ppm. Since a diacetate was formed, ν_{max} (KBr) 1770, 1740 cm^{-1}, a hydroxymethyl group must be present in the molecule. $KMnO_4$ oxidation yielded phthalic acid indicating that only one ring is substituted, but the position of the CH_2OH group could not be assigned from spectroscopic data. Biogenetic evidence makes substitution in 4 position unlikely. To decide whether this group was located in the 2 or 3 position the two anthraquinones in question were synthesized.

1-Hydroxy-2-methyl- and 1-hydroxy-3-methylanthraquinone were first produced,[1] acetylated and then brominated with N-bromosuccinimide. After reacting with $NaOAc–Ac_2O$, the diacetates were isolated and hydrolyzed with $MeOH–H_2SO_4$. The IR, UV and MS of the synthetic 1-hydroxy-2-hydroxymethylanthraquinone were identical to the naturally occurring compound. This product may be identical to that reported from *Digitalis ferruginea*,[2] although the complete structure of the *Digitalis* quinone is not yet known. This is the first report of the occurrence of an anthraquinone in the family Gesneriaceae and is one of the few examples where a naphthaquinone (except for vitamin K_1) and an anthraquinone co-occur in the same plant.

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[2] IMRE, S., TULUS, R. and SENGUEN, J. (1971) *Tetrahedron Letters* (48), 681.

At least two pathways are known for the biosynthesis of anthraquinones in higher plants, the acetate pathway,[3,4] occurring in the Polygonaceae and the shikimate[5,6] or *o*-succinoylbenzoic acid (OSB) pathway[7,8] in the Rubiaceae. Since the Gesneriaceae are taxonomically remote from both the Rubiaceae and Polygonaceae and since at least four different biosynthetic pathways exist, in higher plants, for the formation of the carbon skeleton of naphthaquinones,[9] it was important to determine which pathway was involved in the biosynthesis of this anthraquinone. Table 1 shows the incorporation of potential precursors into 1-hydroxy-2-hydroxymethylanthraquinone. Shikimic acid-[7-^{14}C],[10] OSB-[2,3-^{14}C] and DL-mevalonic acid-[2-^{14}C] are incorporated, thus confirming the suggestion that the OSB-pathway is operative in the biosynthesis of this anthraquinone. In order to determine the specific incorporation of these precursors into the product, the labelled anthraquinone was chemically degraded. 1-Hydroxy-2-hydroxymethylanthraquinone labelled either from shikimic acid-[7-^{14}C] or *o*-succinoylbenzoic acid-[2,3-^{14}C] was oxidized with potassium permanganate. The resulting phthalic acid was isolated and purified to constant specific activity. It contained 101 and 0·2% resp. of the original radioactivity in the anthraquinone molecule. This observation indicates that the OSB-pathway is present in this plant. Similarly, the anthraquinone was labelled from DL-mevalonic acid-[2-^{14}C], oxidized with Ag_2O to 1-hydroxy-2-carboxyanthraquinone, and then decarboxylated with Cu-chromite in quinoline. The resulting 1-hydroxyanthraquinone was purified and contained no radioactivity. This finding suggests again that the hydroxymethyl group of the anthraquinone originates from the *trans*-methyl group of γ,γ-dimethylallylpyrophosphate.[11]

TABLE 1. INCORPORATION OF ^{14}C-LABELLED PRECURSORS INTO 1-HYDROXY-2-HYDROXYMETHYLANTHRAQUINONE IN THE LEAF OF A 1-yr-old PLANT OF *Streptocarpus dunnii* AFTER 24 hr FEEDING

Precursor applied			1-Hydroxy-2-hydroxy methylanthraquinone	
	μmol	Total act. (dpm ×10^6)	Incorp. (%)	Sp. act. (dpm/μmol)
Shikimic acid-(7-^{14}C)	0·184	10·4	0·06	970
o-Succinoylbenzoic acid-(2,3-^{14}C)	0·25	22·2	0·125	31 578
DL-Mevalonic acid-(2-^{14}C)	3·38	43·9	0·16	12 340

The above results show clearly that the biosynthesis of anthraquinones in the Gesneriaceae involves the same or a similar mechanism as reported for rubiaceous-anthraquinones. Thus, the condensation of shikimate or a derivative thereof with an activated

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[4] LEISTNER, E. (1971) *Phytochemistry* **10**, 3015.
[5] LEISTNER, E. and ZENK, M. H. (1967) *Z. Naturforsch.* **22b**, 865.
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[7] DANSETTE, P. and AZERAD, R. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1090.
[8] LEISTNER, E. (1973) *Phytochemistry* **12**, 337.
[9] ZENK, M. H. (1972) *Z. Physiol. Chem.* **253**, 123.
[10] SCHARF, K. H. and ZENK, M. H. (1971) *J. Labelled Comp.* **7**, 525.
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molecular species derived from α-ketoglutarate yields OSB[7] which undergoes ring closure. Subsequent prenylation of the resulting naphthalene would lead to the naphthaquinone dunnione or if ring closure occurred an anthraquinone would be produced.

This is the first report of the occurrence of the OSB-pathway for anthraquinone synthesis outside the Rubiaceae. It is to be expected that the anthraquinones of other Tubiflorae families may also be formed via the OSB-pathway.

EXPERIMENTAL

Isolation and properties of the 1-hydroxy-2-hydroxymethylanthraquinone. The roots (fr. wt 55 g) of the 1-yr-old plants were extracted with Et_2O and alcohol. The solution, evaporated to a small vol. was subjected to preparative TLC of silica gel and developed with solvent I (C_6H_6–HCO_2Et–HCO_2H, 75:24:1). The main product was eluted (Et_2O) and crystallized from MeOH. Yield 156 mg (0·28%); yellow needles; m.p. 212·5–215°. MS: *m/e*; 254 (M^+, 100) 236 (M^+–H_2O, 18), 225 (M^+–COH, 95). IR: ν_{max} (KBr) 3300 (OH), 1672 (non-chelated C=O), 1638 (chelated C=O), 1593 (C=C) cm^{-1}. UV: λ_{max} (MeOH) 222 (ϵ 17 250), 253 (ϵ 29 500), 328 (ϵ 2250), 407 (ϵ 4750) nm. NMR in $(CD_3)_2CO/(CD_3)_2SO$ 4·69 (*s*, 2H), 7·96 (*m*, 6H), 12·81 (*s*, 1H) ppm. The leaves contain only traces of this compound.

Diacetate. Light yellow needles, m.p. 177–180° from EtOH. IR; ν_{max} (KBr) 1768 (C=O), 1740 (C=O), 1678 (C=O), 1590 (C=C) cm^{-1}. MS: *m/e*; 338 (M^+, 1), 296 (M^+-CH_2CO,40), 254 (M^+-2 × CH_2CO), 253 (M^+-2 × CH_2CO,-H,50), 255 (15), 208 (10), 180 (8), 152 (20).

Synthesis of 1-hydroxy-2-hydroxymethylanthraquinone. The synthesis of 1-hydroxy-2-methylanthraquinine was carried out by condensation of phthalic anhydride and *o*-cresol. Crystallization from MeOH gave yellow needles, m.p. 175–177° (lit.[12] 184–185°). IR: ν_{max} (KBr) 1673 (C=O), 1640 (C=O), 1595 (C=C) cm^{-1}. Acetate, m.p. 177–179° (lit.[12] 177–178°). IR: ν_{max} (KBr) 1770 (C=O), 1675 (C=O), 1590 (C=C) cm^{-1}.

Bromination and acetylation. The acetate (109 mg) dissolved in CCl_4 (46 ml) was refluxed with *N*-bromosuccinimide (83·6 mg) and azo-bis-*iso*-butyronitrile (7·8 mg) for 42 hr. The filtrate was evaporated to dryness and the residue boiled 2× with 10 ml H_2O. After filtration a crude product (108 mg) was obtained, which was dissolved in a mixture of Ac_2O (3·3 ml) and dry NaOAc (220 mg). The mixture was stirred (2·5 hr; 110°). The solution was hydrolyzed and the residue purified by chromatography (silica gel GF_{254}, $CHCl_3$–light petrol., 5:2). Yield 18 mg diacetate, m.p. 176–179° from EtOH. The IR spectrum was identical with that obtained from the diacetate of the naturally occurring anthraquinone.

Hydrolysis of the diacetate. The diacetate (8 mg) was refluxed in MeOH–conc. H_2SO_4, 2 ml; (3:0:1) for 2 hr. After purification by chromatography yellow needles (from MeOH) m.p. 212–214° were obtained. Yield 3·6 mg UV, IR and MS were identical with those of the naturally 1-hydroxy-2-hydroxymethylanthraquinone.

Feeding experiments. Streptocarpus dunnii plants were grown in the green house. 1 Plant (1-yr-old), was used for each feeding experiment. A small piece of the midrib was dissected from the leaf and the cut end of the leaf was dipped into the tracer solution. After 25 hr the leaf was extracted with Et_2O, the radioactive 1-hydroxy-2-hydroxymethylanthraquinone isolated and chromatographed for purification on silica gel plates in three solvents (I, II: $CHCl_3$, III: $CHCl_3$–MeOH, 100:1). The incorporation data are summarized in Table 1.

Degradation procedures. Degradation of the labelled anthraquinone to phthalic acid has been described previously.[5]

Oxidation and decarboxylation to 1-hydroxyanthraquinone. The radioactive 1-hydroxy-2-hydroxymethylanthraquinone (4·49 mg, 17·7 μM; 415 dpm/μM) was dissolved in NaOH (50 ml; 2n) and stirred with freshly prepared Ag_2O (250 mg) for 12 hr at 20° and 3 hr at 65°. After acidification with H_2SO_4 (2n) the mixture was extracted with EtOAc, the organic phase exaporated and the residue dried over P_2O_5 and KOH. 1-Hydroxy-2-carboxyanthraquinone was dissolved in freshly distilled quinoline (1 ml) and boiled with copper chromite (12·5) mg; 6 min). The reaction mixture was allowed to cool, diluted with EtOAc (10 ml) and Et_2O (2 ml) and the base extracted with H_2SO_4 (2 × 5 ml; 2n) and HCl (5 ml; 4n). The organic solvent was evaporated to dryness and the residue purified on silica gel plates ($CHCl_3$–light petrol., 1:1). The 1-hydroxyanthraquinone obtained (3·17 μM, yield 18%, spectrophotometrically determined) was inactive.

M.ps were determined on a Kofler hot-stage apparatus and are uncorrected. The NMR spectrum was recorded on Bruker 90 MHz spectrometer and peak positions are given in *d* values (tetramethylsilane as an internal standard). MS were taken on a Varian CH 5 spectrometer.

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