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GERMICHRYSONE, A HYDROANTHRACENE DERIVATIVE FROM SEEDLINGS OF *CASSIA TOROSA**

SHUHICHI TAKAHASHI,† MICHIO TAKIDO,† USHIO SANKAWA‡ and SHOJI SHIBATA‡
†Department of Pharmacy, College of Science and Technology, Nihon University, Chiyoda-ku, Tokyo 101, Japan;
‡Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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Key Word Index—*Cassia torosa*; Leguminosae; seedlings; germination; germichrysone; hydroanthracene; 1-oxo-3,8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracene.

Abstract—From seedlings of *Cassia torosa* chrysophanol, physcion, emodin, torosachrysone and a new yellow pigment, germichrysone, were isolated. The structure of germichrysone was established as 1-oxo-3,8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracene. A preliminary feeding experiment with radioactive malonate showed that germichrysone is biosynthesized *de novo* in the seedlings.

INTRODUCTION

In a previous paper, we reported the isolation and characterization of several anthraquinones 1–5 and a hydroanthracene derivative, torosachrysone 6, from a purgative drug, the seeds of *Cassis torosa* Cavanilles (Japanese name "Habuso") [1]. During these studies, we found that seedlings of this plant showed a markedly different pattern of secondary metabolites by comparison with the seeds. Changes in secondary metabolites were investigated throughout the course of germination and revealed that the glucoside 5 disappeared rapidly and, at the same time, a new yellow pigment appeared as the characteristic constituent of the seedlings. This compound is named germichrysone, as it is found only in the young seedling†.

RESULTS AND DISCUSSION

The C_6H_6 extracts of the fresh seedlings were chromatographed on Si gel and after elution of fractions containing chrysophanol (1), physcion (2), emodin (3) and unidentified compounds‡, a fraction containing torosachrysone (6) and germichrysone was eluted. Germichrysone, finally separated from torosachrysone (6) by re-chromatography, was obtained as yellow needles mp 198°. High resolution MS showed the molecular formula, $C_{15}H_{14}O_4$, and major fragment ions were observed at *m/e* 240(M^+-H_2O) and *m/e* 225(M^+-H_2O-CO). The similarity in the chromophores of germichrysone and torosachrysone (6) as well as synthetic hydroanthracene derivatives (7,8) [2] was readily established by comparison of their UV and IR spectra. An intense band at 1630 cm^{-1} revealed the presence of a chelated carbonyl, while the UV maxima at 300 and 311 nm showed that germichrysone possesses a napthalene nucleus. The PMR spectrum of germichrysone gave a multiplet (1 H) at δ4.28, which is attributed to the proton ($H_{(3)}$) on a carbon bearing a secondary OH group. Spin decoupling experiments with the Me ether (10) established the location of the secondary OH group. Irradiation at the frequency of the proton ($H_{(3)}$, δ4.49, m) collapsed two multiplets ($H_{(2)}$ and $H_{(4)}$) at δ2.87 and 3.24 into two doublets, and on irradiation at the centre of the two multiplets (δ3.06) the multiplet ($H_{(3)}$) at δ4.49 changed to a singlet. The results clearly indicated that both of the two $-CH_2-$ groups are located adjacent to the secondary OH group. The presence of two *m*-coupled aromatic protons ($H_{(5)}$ and $H_{(7)}$) and an aromatic Me indicated that the dispositions of these groups on the aromatic ring of germichrysone would be identical to that of the co-occurring anthraquinones (1, 2 and 3).

All these indications and biogenetical considerations led to structure 9 for germichrysone. This structure was also supported by the following findings. Treatment of germichrysone (9) with alcoholic NaOH gave chrysophanol (1), and acetylation with Ac_2O and C_5H_5N yielded 1,8,9-triacetoxy-3-methyanthracene (11) which on further treatment with alcoholic NaOH afforded chrysophanol (1). In contrast, the Me ether (10) did not give chrysophanol (1) under alkaline condition, since the OH group required to generate a quinoid system was blocked by a Me group. As a final proof for the structure, germichrysone (9) was synthesized from emodinanthrone by catalytic hydrogenation. The MS and PMR spectra of the synthetic product were identical to those of the natural product.

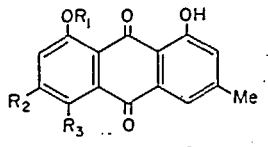
The appearance of germichrysone (9) coincides with the disappearance of the anthraquinone glucoside (5), which is a main constituent of the seeds and suggests that germichrysone (9) is derived from the glucoside (5).

* Part 9 in the series "Studies of the Constituents of Purgative Crude Drugs". For Part 8 see ref. [1].

† Recently the callus of *C. torosa* was also found to produce germichrysone (9).

‡ A study dealing with these compounds will be reported in a separate paper.

When seeds were germinated in a solution of malonate-[2-^{14}C], significant radioactivity (incorporation ratio 0.16%) was found in germichrysone (9) isolated from the seedlings. This result clearly indicates that germichrysone (9) is not derived from the anthraquinones originally contained in the seeds, but is a product of *de novo* biosynthesis in the seedlings.



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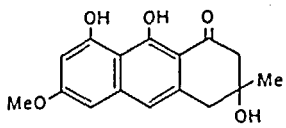
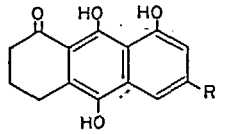
2 R_1=H R_2=OMe R_3=H
3 R_1=H R_2=OH R_3=H
4 R_1=H R_2=OMe R_3=OH
5 R_1=*Glu-Glu* R_2=OMe R_3=H



6



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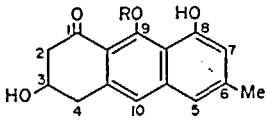

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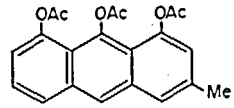
10 R=Me



11

EXPERIMENTAL

Plant material was obtained from the Drug Plant Garden of the College of Science and Technology, Nihon University. The known compounds were identified by TLC, IR and mmp. PMR were recorded using TMS as internal standard. Column chromatography was carried out on Si gel. Silicic acid (Mallinkrodt) or Si gel which has been washed with N/2 oxalic acid and reactivated is essential for the separation of the compounds described in this paper.

Extraction and isolation. Seeds (1 kg) were sterilized with 5% NaOCl soln and germinated in the dark at 29° on a wet cotton wool bed. After 1 week, yellow seedlings 2–3 cm high were harvested and extracted with hot C_6H_6 (4 × 6 l). The C_6H_6 extracts, upon chromatography with C_6H_6–EtOAc (4:1), gave 7 fractions. Fraction 1 was re-chromatographed with petrol–MeOH (7:3) to give chrysophanol (1) (trace) and physcion (2) (270 mg). Fraction 2 gave emodin (3) (20 mg) and fraction 5, upon rechromatography with petrol –C_6H_6–EtOAc (7:1:2) gave 2 fractions. The faster eluting fraction gave torosachrysone (6) (100 mg) and the other gave germichrysone (9) (100 mg). Several unidentified compounds were obtained from fractions 3, 4, 6 and 7.

Germichrysone (9) was recrystallized from C_6H_6 to give yellow needles mp 196°, $[\alpha]_D^{17°}$ −38.5° (dioxane; *c* 0.52). High resolution MS: Found 258.0862 ($C_{15}H_{14}O_4$); UV $\lambda_{max}^{dioxane}$ nm (log ε): 271 (4.77), 300 (3.64), 311 (3.66), 402 (3.94); IR ν_{max}^{KBr} cm^{-1}: 3350, 1630, 1607, 1587; MS 70eV *m/e* (rel. int.): 258 (M^+; 60), 240 (M^+–H_2O; 100), 225 (M^+–H_2O–Me; 25), 212 (M^+–H_2O–CO; 24); PMR (60 MHz, $CDCl_3$): δ2.00 (1H, *br s*, –CH–OH), 2.32 (3H, *s*, –Me), 2.80 (2H, *m*, –CH_2–), 3.00 (2H, *m*, –CH_2–), 4.28 (1H, *m*, –CH–OH), 6.70 (1H, *s*, Ar–H), 7.02 (2H, *br. s*, Ar–H × 2), 9.70 (1H, *s*, OH), 15.72 (1H, *br s*, OH).

Germichrysone monomethyl ether (10). 9 (50 mg) was methylated with CH_2N_2. The reaction product was purified by column chromatography using C_6H_6–EtOAc (4:1) as eluant. 10 was crystallized from MeOH as pale yellow needles (18 mg) mp 196–200°, IR ν_{max}^{KBr} cm^{-1}: 1680; MS 70eV *m/e*: 272 (M^+); PMR (60 MHz, $CDCl_3$): δ3.24 and 2.87 (2H × 2, *m* × 2, –CH_2– × 2), 2.37 (3H, *s*, Me), 4.03 (3H, *s*, OMe), 4.49 (1H, *m*, –CH–OH), 6.77 and 7.03 (1H × 2, *br s* × 2, Ar–H × 2), 7.36 (1H, *s*, ArH).

Chrysophanol from germichrysone (9). 9 (10 mg) was dissolved in a mixture of 5% NaOH (5 ml) and MeOH (5 ml) and the soln heated at 50°, acidified and extracted with C_6H_6. The C_6H_6 extract was recrystallized from MeOH to give 1 (5 mg).

1,8,9-Triacetoxy-3-methylanthracene (11) 9 (10 mg) was dissolved in a mixture of Ac_2O (0.5 ml) and C_5H_5N (2–3 drops). After standing 18 hr at 20°, the reaction mixture was worked up as usual. The product was repeatedly purified by column chromatography using C_6H_6–$CHCl_3$ (1:1). Recrystallization from MeOH gave colourless crystals (4 mg) mp 250–252°. MS 70eV *m/e* (rel. int.): 366 (M^+; 21), 324 (M^+–Ac; 20), 282 (M^+–Ac × 2; 42), 240 (M^+ –Ac × 3; 100); PMR (60 MHz, $CDCl_3$): δ2.30 (3H, *s*, Me), 2.42 (6H, *s*, Ac × 2), 2.50 (3H, *s*, Ac), 7.06–8.73 (6H, *m*, Ar–H × 6). On treatment with MeOH–NaOH, 11 yielded 1.

Synthesis of germichrysone (9). The catalytic hydrogenation of emodinanthrone gave anomalous results and compositions of the products according to the batches of varied PtO_2. The use of a large amount of catalyst is essential for this reaction. Emodinanthrone (300 mg) was catalytically hydrogenated in EtOAc–EtOH–C_6H_6 (5:5:3) PtO_2 (100 mg) as a catalyst. Repeated PLC (0.5 mm layer of Si gel impregnated with N/2 oxalic acid) and recrystallization from MeOH gave yellow needles (60 mg) mp 195°. TLC, UV and PMR were identical with those of the natural product, but the IR spectra showed slight differences probably due to their difficult crystal forms.

Feeding experiment. A flask containing seeds (5 g) and the soln (99 ml) of diEt malonate [2-^{14}C] (6.58×10^5 dpm) were shaken on a rotatory shaker (200 rpm) for 3 days in the dark at 25°. Germinated seedling were filtered and extracted with EtOAc to obtain germichrysone (9). Purification by column chromatography followed by PLC gave crystalline germichrysone (9) (1.1×10^4 dpm, 0.16% incorporation).

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REFERENCES

1. Takido, M., Takahashi, S., Masuda, K., and Yasukawa, K. (1976) *Lloydia* (in press).
2. Seo, S., Sankawa, U., Ogihara, Y., Iitaka, Y., and Shibata, S., (1973), *Tetrahedron* **29**, 3271.