Chromatograph: 5% OV-1 on Chromosorb 60/80 column, temp programmed from 100° to 250° at 10°/min, FID.

Isolation of methyl esters from L. ponderosus. Mycelial mats from 4 flasks of a 5-week-old culture were separated from the medium by decanting and ground in a Waring blender with MeOH. After filtering, MeOH was evaporated and residue extracted with Et_2O (mycelial dry wt: 3.2 g). The medium (pH 4.3) was extracted with Et_2O and pooled together with the Et_2O extract from the mycelial mat. A small sample of this extract and the Et_2O extract of the medium acidified to pH 2 were used for TLC, PC and GLC in the detection of phenolic compounds. The remaining total Et_2O extract was passed through a silicic acid column with C_6H_6–Me_2CO (19:1) as eluting solvent to isolate a mixture of the methyl esters of cinnamic and benzoic acids. These methyl esters were then separated on a Sephadex LH-20 column. The gel was swollen in MeOH satd with *n*-heptane and the eluting solvent was *n*-heptane satd with MeOH. The appearance of each compound in the eluate was monitored by UV. Methyl cinnamate was the first compound collected (0.9 mg). Its identity was proved by UV, GLC and TLC. UV. λ_{max}^{MeOH} (log ε) 217, 222,275 nm (4.14, 4.13, 4.16). The second fraction consisted of a very small amount of methyl *p*-methoxybenzoate (λ_{max}^{MeOH} 254 nm) and *cis* and *trans* methyl *p*-methoxycinnamate as determined by UV and GC. The third fraction contained *trans* methyl *p*-methoxycinnamate (108 mg), mp 87–88°, λ_{max}^{MeOH} (log ε) 225,291 *sh*, 298 *sh*, 309 nm (4.13, 4.16, 4.16, 4.17). Its *trans* configuration was determined by NMR which gave a coupling constant value of 16 Hz for the methylene protons. It also showed the same retention time as a standard on GLC. Methyl isoferulate (8 mg) was eluted from the column with MeOH satd with *n*-heptane, and identified by UV, TLC and NMR. UV λ_{max}^{MeOH} (log ε) 220, 242, 294, 324 nm (4.13, 4.13, 4.14, 4.14).

Enzyme assay. Enzyme assay for phenolic *O*-methyltransferase was carried out as described for *L. lepideus* [3].

Acknowledgements—We thank Dr. R. J. Bandoni, Dept. of Botany, University of B.C., for cultures of *L. edodes* (UBC 767) and *L. lepideus* (UBC 718), Dr. R. S. Smith, Western Forest Products Laboratory, Vancouver, B.C. for *L. vulpinus* (177A), Dr. J. H. Ginns of Agriculture Canada, Ottawa, for *L. cochleatus* (DAOM 22534) and *L. kauffmanii* (DAOM 10722, 11660, 17180) and Dr. J. G. Palmer, USDA, Madison, Wisconsin, for *L. tigrinus* (RLG-9953-Sp), *L. sulcatus* (OKM-8302-Sp) and *L. ponderosus* (OKM-3120-S). We also thank Elizabeth A. Graham for technical assistance, Dr. E. Rodriguez for NMR spectra and the National Research Council of Canada for financial support.

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Phytochemistry, 1977, Vol. 16, pp. 291–293, Pergamon Press, Printed in England.

TWO NEW COUMARINS IN *BOENNINGHAUSENIA ALBIFLORA*

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(*Received* 26 *July* 1976)

Key Word Index—*Boenninghausenia albiflora*; Rutaceae; (*E*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one; (*Z*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one.

Abstract—Two new isomeric coumarins were isolated from leaves of *Boenninghausenia albiflora* Reichb. Their structures were elucidated as (*E*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one and (*Z*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one.

INTRODUCTION

The occurrence of many coumarins in *Boenninghausenia albiflora* has been reported [1–7], among which, a dimeric coumarin, matsukaze lactone [2], nodakenetin acetate [3] and 3-(1,1-dimethyl allyl)-xanthyletin [4] were reported to be novel.

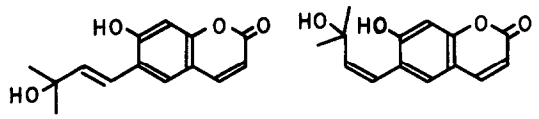
In the course of our studies on the fragrant components in the essential oil of *B. albiflora*, two compounds were conspicuous because of their strong fluorescence under UV irradiation and higher polarities on TLC. The isolation and structural elucidation of these compounds has now revealed them to be (*E*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one and (*Z*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one. Some other coumarin derivatives which were new in this essential oil were also identified.

RESULTS AND DISCUSSION

Structure of compound 1

From the ether extract of the steam distillate of the plant a crystalline precipitate separated out on evaporation of the solvent. It had a mp 159–160° after recrystallization from acetone–benzene (compound **1**). It gave a

single UV fluorescent spon on TLC developed with several solvent systems.



1 2

Scheme 1

By high resolution MS analysis, the formula of **1** was estimated as $C_{14}H_{14}O_4$ and the following fragmentation was shown: MS (probe) *m/e* (rel. int.): 246 M^+ (3), 231 (M^+-Me; 4), 228 (M^+-H_2O: 18), 213 (M^+-H_2O,Me; 100), 185 (M^+-H_2O,Me,CO; 36).

The IR spectrum of **1** showed absorptions at 3370 and and 3200 (—OH), 1698, 1617 and 1571 (coumarin system), 1391 and 1380 (—$(Me)_2$) and 966 cm^{-1} (*trans*-disubstituted alkene). It gave positive results to both the ferric chloride–pyridine test (blue) and Denigès test [8]. Trimethylsilylation of **1** [9] introduced two TMSi groups, which was confirmed by high resolution MS analysis: $C_{20}H_{30}O_4Si_2$ (M^+ *m/e* at 390).

These results showed that **1** had two hydroxyl groups, one of which was phenolic and the other was tertiary. In alkaline solution it showed blue fluorescence under visible light, indicating a 7-hydroxy coumarin derivative for the structure of **1** [10]. The UV spectrum λ_{max} nm (log ε): 256 (4.43), 344 (4.08) was also consistent with a substituted coumarin structure.

The NMR spectrum of **1** revealed the nature of the side-chain. The signals corresponding to the C-3 and C-4 protons appeared as an AB quartet [δ 6.18 (1H, J = 9.5 Hz), δ 7.84 (1H, J = 9.5 Hz)]. The signals at δ 7.70 (1H, *s*) and 6.77 (1H, *s*) were attributable to the C-5 or C-6 proton and the C-8 proton respectively. The remaining signals, arising from the side-chain (HO—C_5H_8) protons, were a singlet at δ 1.38 (6H) corresponding to two methyl groups and an AB quartet [δ 6.89 (1H, J = 16 Hz), δ 6.49 (1H, J = 16 Hz)] due to the *trans* olefinic protons C-1′ and C-2′.

Dehydrative cyclization of **1** afforded xanthyletin (8,8-dimethyl-2*H*,8*H*-benzo[1,2-b:5,4-b′]dipyran-2-one), indicating that **1** has the substituent group at the C-6 position.

Thus the structure of compound **1** was determined as (*E*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one.

Structure of compound **2**

Compound **2** was obtained by repeated HPLC of the essential oil. It had a mp 139–140.5° after recrystallization from acetone–benzene and gave a single UV fluorescent spot on TLC with several solvent systems.

The molecular formula of compound **2** was estimated as $C_{14}H_{14}O_4$ by high resolution MS. The following fragmentation peaks were shown: MS (probe) *m/e* (rel. int.): 246 M^+ (1.2), 231 (M^+-Me; 2), 228 (M^+-H_2O; 9), 213 (M^+-H_2O,Me; 100), 185 (M^+-H_2O,Me,CO; 26). The fragmentation pattern was almost entirely identical with that of **1**.

Trimethylsilylation of **2** also resulted in the introduction of two TMSi groups, which was confirmed by high resolution MS analysis: $C_{20}H_{30}O_4Si_2$ (M^+ *m/e* at 390).

The IR spectrum of **2** showed absorptions at 3400 (—OH), 1700, 1616 and 1570 (coumarin system) and 1392 cm^{-1} (—$(Me)_2$).

The presence of a tertiary hydroxyl group (Denigès test) and a phenolic hydroxyl group (ferric chloride–pyridine test) was suggested, and in alkaline solution it showed blue fluorescence under visible light (7-hydroxy coumarin derivatives). Dehydrative cyclization of **2** also afforded xanthyletin.

The UV spectrum of **2** was similar in shape to that of **1** but shifted to lower wavelengths; λ_{max}nm (log ε): 249 (4.29), 332 (4.13). The IR spectrum of **2** showed the absence of absorptions corresponding to a *trans* olefinic double bond. These results suggested that **2** might be the *cis* isomer of **1**.

In the NMR spectrum of **2**, the signals corresponding to the C-3 and C-4 protons appeared as an AB quartet [δ 6.16 (1H, J = 9.5 Hz), 7.80 (1H, J = 9.5 Hz)]. The signals at δ 7.62 (1H, *s*) and 6.73 (1H, *s*) were attributable to the C-5 proton and the C-8 proton respectively. The remaining signals, arising from the side-chain protons, were a singlet at δ 1.35 (6H) corresponding to two methyl groups, and an AB quartet [δ 6.31 (1H, J = 12.5 Hz), 5.88 (1H, J = 12.5 Hz)] due to the *cis* olefinic protons at C-1′ and C-2′.

Thus the structure of compound **2** was determined as (*Z*)-7-hydroxy-6-(3-hydroxy-3-methyl-1-butenyl)-2*H*-1-benzopyran-2-one.

Further support for the stereochemistry of the double bond in **1** and **2** was obtained by the dehydrative cyclization of them with zinc chloride at room temperature. Compounds **1** and **2** afforded xanthyletin (18% and 52% respectively) after 1 hr reaction.

Isolation of bergapten, chalepensin and xanthyletin.

Another crystalline compound was isolated from the essential oil after removal of **1**. This was identified as bergapten by mp, MS, IR, UV and NMR. Chalepensin and xanthyletin were also isolated by HPLC of the essential oil. The spectral data of isolates were both identical with those reported by Morita *et al.* [11] and Kozawa *et al.* [6]. These three compounds have already been identified in this plant [1,3,6].

EXPERIMENTAL

B. albiflora was gathered at the mountainside in Kanagawa, Japan in September, 1975, and was identified by Emeritus Professor Dr. Fumio Maekawa (Tokyo University).

Mp's are uncorrected. UV spectra were measured in dioxane. IR spectra were recorded for KBr discs. NMR spectra in CD_3COCD_3 were determined at 100 MHz using TMS as internal standard. MS were determined using a voltage of 70 eV.

Isolation of compound **1**. The aerial part of the plant (2.3 kg) was steam distilled. The distillate was extracted with ether. The ether was evaporated using a distillation column packed with helipak. From the residual oil (2.79 g) after being allowed to stand at room temp, precipitated a white mass. Recrystallization from McCOMe–C_6H_6 gave colourless platelets, mp 159–160°. High resolution MS, *m/e* 246 (M^+) $C_{14}H_{14}O_4$. (Found: 246.0866. Calcd: 246.0890). High resolution MS of the TMSi derivative, *m/e* 390 (M^+) $C_{20}H_{30}O_4Si_2$ (Found: 390.1677. Calcd: 390.1680).

Cyclization of **1**. Compound **1** (15 mg) was suspended in C_6H_6 (10 ml). Silicic acid (LiChrosorb SI 60) (350 mg) was added and refluxed for 4 hr. The organic layer was separated from the silicic acid and evaporated *in vacuo* to give a white crystalline substance (4.3 mg), which had a mp 130.5–131.5° and gave a single fluorescent spot on TLC. This substance was identified as xanthyletin by mp, mmp, IR, UV and NMR.

Isolation of compound **2**. The essential oil was chromatographed on HPLC using a 50 × 0.8 cm LiChrosorb SI 60 (Merck) column. The eluant was MeCOMe–C_6H_6 (2:8), at a flow rate of 145 ml/hr. A Pye LCM2 hot wire detector was used. The fractions which contained a substance eluted at retention time 13 min were combined. After evaporation of the solvent a crystalline substance was obtained (19.5 mg), Recrystallization from MeCOMe–C_6H_6 gave colourless platelets, mp 139–140.5°. High resolution MS, *m/e* 246 (M^+) $C_{14}H_{14}O_4$. (Found: 246.0887, Calc. for 246.0890). High resolution MS of the TMSi derivative *m/e* 390 (M^+) $C_{20}H_{30}O_4Si_2$. (Found: 390.1679. Calcd: 390.1680).

Cyclization of **2**. Cyclization of compound **2** was carried out by the same methods described above to give xanthyletin (6.0 mg) (mp 130.8–131.8°).

Cyclization of **1** *and* **2** *with* $ZnCl_2$. Each of **1** (0.23 mg) and **2** (0.23 mg) was suspended in C_6H_6 (2 ml) and $ZnCl_2$ (4 mg) was added, and the mixture was stirred at room temp. An aliquot (50 µl) of the organic layer was taken out every 10 min and chromatographed on HPLC using a 30 × 0.3 cm LiChrosorb SI 60 (Merck) column with EtOAc–C_6H_6 (5:95) as an eluant, at a flow rate of 35 ml/hr. CECIL CE212 (UV 348 nm) was used as a detector. The retention time of xanthyletin was 5.15 min. The quantity of the product was estimated from the peak height and the calibration curve given by authentic xanthyletin.

Isolation of bergapten, chalepensin and xanthyletin. Leaving the essential oil in a refrigerator for a month, after the removal of **1**, gave bergapten (11.4 mg). Recrystallization from EtOH gave white needles, mp 187.5–189°. On HPLC of the residual essential oil, hexane eluates afforded chalepensin (110 mg). It was recrystallized from EtOH–H_2O mp 87.9–88.2°. The subsequent fractions eluted with C_6H_6 yielded xanthyletin (54.4 mg). It was recrystallized from C_6H_6–hexane mp 131–132°.

Acknowledgements—The authors wish to thank Emeritus Professor Dr. F. Maekawa (Tokyo University) for the identification of the plant material and also to Dr. T. Fujimori of this institute for the help of collecting the plant material.

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Phytochemistry, 1977, Vol. 16, p. 293, Pergamon Press, Printed in England.

A PRENYLATED CHALKONE FROM *MILLETIA OVALIFOLIA*

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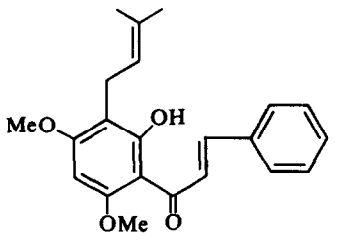
(*Received* 20 *August* 1976)

Key Word Index—*Milletia ovalifolia*; Leguminosae; A new prenylated chalkone; ovSlichalkone; 2′-hydroxy-3′-C-prenyl-4′,6′-dimethoxy chalkone).

In continuation of our earlier work [1], further examination of the seeds of *Milletia ovalifolia* led to the isolation of an additional chalkone designated as ovalichalkone. It was assigned structure (**1**) based on spectral and synthetic evidence. mp 123–24° yellow needles, molecular formula $C_{22}H_{24}O_4$ M^+ (352), +ve Gibbs test. λ_{max}^{MeOH} nm 345 (log ε 4·49). IR ν_{max}^{KBr}: 1630, 1580, 1470, 1410, 1330, 1220, 1120, 980, 780 cm^{-1}. PMR (δ values solvent $CDCl_3$): gem dimethyl allyl group is shown by the following peaks. 1·8(*d*, 6H, *J* = 6 Hz, $C\underline{H}_3)_2C$=); 3.35 (*m*, 2H, $-C\underline{H}_2$—CH=C); 5.4 (*m*, 1H, —$C\underline{H}$=$C(Me)_2$. Two methoxyl groups shown by peaks at 4.0 (*s*, 3H, —OMe) and 3.95 (*s*, 3H, —OMe); aromatic protons and α, β-protons are shown by peaks at 6.1 (*s*, 1H, H-5′); 7.55 (*m*, 5H, H-2, 3, 4, 5, 6); 7.95 (*s*, 2H, H-α, β); chelated hydroxyl at 14.6 (*s*, 1H, —OH at 2′) (D_2O exchangeable). The mass fragments at 337 (99%) (M-15); 321 (14.3) (M-31); 309 (99) (M-43); 297 (85.3) M-55); 275 (25.3) (M-77) and at 248 (14.3) (M-104=a); 233 (98) (a-15); 205 (77) (a-43); 193 (99) (M-55-104); 131 (44) (cinnamoyl); 103 (66), agreeing with the structure **1** for ovalichalkone. This was confirmed by synthesis from 5-*C*-prenyl-2,4-di-*O*-methyl-phloroacetophenone mp 113–114° (lit. [2] mp 113°C); UV λ_{max}^{MeOH} nm 290 (log ε 4.33). IR ν_{max}^{KBr} 1620, 1590, 1460, 1415, 1380, 1360, 1230, 1180, 1130, 890 cm^{-1}. PMR (δ values solvent $CDCl_3$);



1.7 (*d*, 6H, *J* = 6 Hz ($C\underline{H}_3)_2C$=); 2.6 (*s*, 3H, —$COC\underline{H}_3$); 3.3 (*m*, 2H —$C\underline{H}_2$—CH=$C(Me)_2$); 3.9 (*s*, 6H, 2 —OMe); 5.2 (*m*, 1H, —CH_2 $C\underline{H}$=$C(Me)_2$); 6.0 (*s*, 1H, H-3); 14.4 (*s*, 1H, chelated —OH). The above ketone on condensation with benzaldehyde under alkaline conditions yielded a chalkone which was identical with the natural sample (co-TLC, mmp and co-IR spectra in KBr).

Acknowledgements—The authors are grateful to the Director, CIBA-GEIGY Research Centre, Bombay for the mass spectra and to the Council of Scientific and Industrial Research (India) for financial assistance to one of them (R.K.G.).

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