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4-HYDROXY-5-METHYLCOUMARIN DERIVATIVES FROM *DIOSPYROS KAKI* THUNB AND *D. KAKI* VAR. *SYLVESTRIS* MAKINO; STRUCTURE AND SYNTHESIS OF 11-METHYLGERBERINOL

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Key Word Index—*Diospyros kaki* Thunb; *D. Kaki* Thunb var. *sylvestris* Makino; Ebenaceae; 4-hydroxy-5-methylcoumarin derivatives; dicoumarol derivatives; gerberinol; 11-methylgerberinol.

Abstract—Two 4-hydroxy-5-methylcoumarin derivatives have been isolated from the roots of *Diospyros kaki* Thunb and *D. kaki* Thunb var. *sylvestris* Makino. One was shown to be identical to gerberinol, previously isolated from the Compositae plant *Gerbera lanuginosa*. The other was found to be a new natural product whose structure, 11-methylgerberinol, was proved by spectral analysis and further confirmed by synthesis.

INTRODUCTION

Naturally occurring coumarins with an oxygen atom at C-4 and a methyl substituent at C-5 were few until 1978 but the number has now exceeded 100 and most of them have been isolated from the Compositae [1]. Naphthoquinones and related compounds have been isolated from the root extracts of Ebenaceae plants *Diospyros kaki* Thunb and *D. kaki* Thunb var. *sylvestris* Makino [2]. While many could be characterized, two compounds, now designated as A and B, were available in minute quantities and could not be identified. These two compounds have been now characterized as dicoumarol derivatives gerberinol (**1**) and 11-methylgerberinol (**2**) by spectral analysis. While **1** was previously isolated from a Compositae plant [3], **2** is a new natural product and the assigned structure is further confirmed by synthesis.

RESULTS AND DISCUSSION

Compound A, $C_{21}H_{16}O_6$ (EIMS), mp 267–269°, showed UV (λ_{max} 240, 286, 320sh nm) and IR bands ν_{max} 3030, 1648, 1599 cm^{-1}) typical of a bis-4-hydroxycoumarin [4]. The ^1H NMR spectrum showed signals at δ2.80 (6H, *s*, aromatic methyls), 3.77 (2H, *s*, CH_2 attached to quaternary carbons), 6.9–7.5 (6H, *m*, typical of a 1,2,3-trisubstituted benzene ring) and two exchangeable protons at δ11.7 (-OH). The high resolution EI-mass spectrum showed a $[M]^+$ peak at 364.094 corresponding to the molecular formula $C_{21}H_{16}O_6$. The peak at *m/z* 134 further suggested that compound A was a bis-coumarol derivative with a methyl substitution either at C-5 or C-8. A survey of the literature indicated that the spectral data and the mp of compound A corresponded exactly to the data reported for gerberinol (**1**) (mp 263–65°), previously isolated from *Gerbera lanuginosa* Benth. A direct spectral comparison (IR, UV, ^1H NMR) unambiguously established the identity.

The second compound B, $C_{22}H_{18}O_6$ (EI mass spectrometry), mp 210–217°(ethyl acetate), exhibited spectral data UV (λ_{max} 246, 295, 305 and 320 nm), IR (ν_{max}, 1660, 1640, 1595 cm^{-1}) characteristic of bis-4-hydroxycoumarin. The infrared bands at 1383 and 1365 cm^{-1} indicated the presence of methyl groups. The ^1H NMR spectrum was strikingly similar to that of compound A (gerberinol, **1**), the major difference being the replacement of one of the C-11 hydrogens by a methyl group (δ1.85, 3H, *d*, J = 7 Hz, 4.65, 1H, *q*, J = 7 Hz). Structure **2** was therefore assigned to compound B. Its high-resolution EI mass spectrum, showed, in addition to the $[M]^+$ peak at *m/z* 378.112 ($C_{22}H_{18}O_6$ requires 378.110), significant peaks at *m/z* 202, 176, 135, (100%), 134 (96%) and 106. The observed fragment ions are fully consistent with structure **2**.

The assigned structure **2** for compound B was further confirmed by a synthesis with 59% yield by refluxing of an ethanolic solution of 4-hydroxy-5-methylcoumarin **3** (prepared by a new synthetic route [5]) and acetaldehyde for 5 min. The synthetic product **2** was identical in all respects to natural **2** (IR, UV, ^1H NMR). In addition, we have now recorded the ^{13}C NMR spectrum and the chemical shifts are fully consistent with the structure

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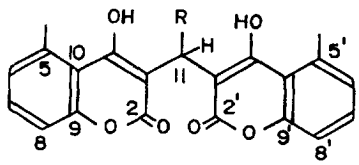

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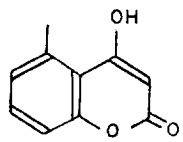
1 H

2 Me



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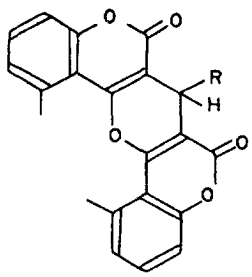
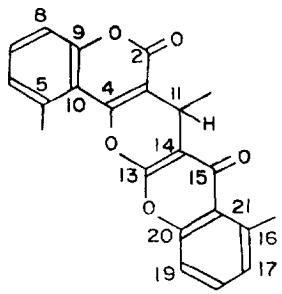


R

4 H

5 Me



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2 and the assignments are given in the experimental section.

Natural 11-methylgerberinol (2) on treatment with acetic anhydride and sulphuric acid gave needles, mp 283–284° (MeOH–$CHCl_3$); $C_{22}H_{16}O_5$ $[M]^+$ 360.098; UV λ_{max} 277, 300 nm; IR ν_{max} 1715, 1670, 1640, 1605, 1410, 1313, 1260 cm^{-1}. Its EI mass spectrum showed, in addition to the $[M]^+$, a base peak at *m/z* 345 $[M-15]^+$ and ^1H NMR spectrum did not show any signals due to acetate methyls and the molecular formula suggested the formation of an anhydro compound. Interestingly, Sengupta and co-workers [3] observed that gerberinol (1), when treated with Ac_2O pyridine gives an anhydro compound (4) and this structure assignment was based on mechanism and spectral analysis (^1H NMR and ^{13}C NMR). Structure 5 therefore looked likely for the anhydro derivative obtained by us from 11-methylgerberinol (2). However, ^1H NMR and ^{13}C NMR data of the anhydro 11-methylgerberinol were not consistent with structure 5. We have assigned structure 6 to this product, especially after noting two different types of carbonyl groups (singlets at δ160.4 and 178.7). The assignments of the ^1H and ^{13}C NMR spectra are given in the experimental section.

EXPERIMENTAL

Mps: uncorr. UV and IR spectra were recorded in EtOH and as KBr pellets, respectively.

Isolation of compound A (gerberinol, 1). The dried and chipped roots (300 g) of *D. kaki* var. *sylvestris* collected at Kiyosumi, Chibs, were extracted with MeOH and partitioned between hexane–water H_2O. The hexane extract on chromatography over silica gel yielded, along with lupeol (60 mg) and 7-methyl juglone (20 mg), compound A (20 mg), mp 267–269° (benzene). UV λ_{max} nm (log ε): 240 (4.31), 286 (4.30) and 320sh (4.10); IR ν_{max} cm^{-1}, 3030, 1648, 1599, 1560, 1350, 1300, 1228, 1118 and 790; ^1H NMR (60 MHz, $CDCl_3$): δ2.80 (6H, *s*), 3.77 (2H, *s*), 6.9–7.5 (6H, 1, 2, 3 trisubstituted benzene): EIMS (70 eV) *m/z*: 364.094 $[M]^+$ cal. for $C_{21}H_{16}O_6$ 364.095), base peak at *m/z* 134.

Isolation of compound B (11-methylgerberinol, 2). The dried and milled roots of *D. kaki* collected at Tokyo (2.0 kg) were extracted with $CHCl_3$ for 20 days at room temp. and the extract (16 g) was chromatographed over silica gel (1.6 kg). Elution with benzene gave a solid (90 mg) which was repeatedly crystallized from EtOAc, mp 210–217°, UV λ_{max} nm (log ε): 246 (4.12); 295 (4.41), 305 (4.34), 320 (4.12); IR ν_{max} cm^{-1}: 1660, 1640, 1595, 1550, 1325, 1309, 1224, 789 and 745, ^1H NMR (60 MHz, $CDCl_3$); δ1.85 (3H, *d*, J = 7 Hz), 2.79 (6H, *s*), 4.65 (1H, *q*, J = 7 Hz), 7.0–7.5 (6H, two 1, 2, 3-trisubstituted benzene moieties), 11.72 (1H, *bs*), 12.45 (1H, *s*); EIMS (70 ev) *m/z* (rel. int.) 378.112 $[M]^+$, 202, 176, 135 (100), 134 and 106.

Reaction of compound B with Ac_2O–H_2SO_4 To compound B (50 mg) in Ac_2O (4 ml) was added a few drops of conc. H_4SO_4 and the mixture was warmed to produce a clear soln. It was left at room temp. for 24 hr after which time a solid separated out. It was filtered and crystallized from MeOH–$CHCl_3$ (46 mg); mp 283–284, UV λ_{max} nm (log ε); 277 (4.09), 300 (4.33) and 333sh (3.82);

IR ν_{max} cm^{-1}: 1715, 1690, 1640, 1605, 1480, 1410, 1373, 1260, 1170, 1030, 795 and 780; ^1H NMR (60 MHz, $CDCl_3$): δ1.49 (3H, *d*, J = 7 Hz), 2.87 (3H, *s*), 2.89 (3H, *s*), 4.26 (1H, *q*, J = 7 Hz), 7.1–7.52 (6H, *m*, H-Ar); EIMS (70 eV) *m/z* (rel. int.) 360 $[M]^+$, 345 (100).

Preparation of 11-*methylgerberinol* (**2**) Acetaldehyde (5 ml) was added to a boiling soln of 4-hydroxy-5-methylcoumarin (**3**) (75 mg) in aq. EtOH (3 ml) and the mixture refluxed for 5 min. Removal of excess acetaldehyde and EtOH yielded a solid which on repeated crystallization from MeOH gave a crystalline solid **2** (48 mg, 59%), mp 218° (lit. [1] 210–217°), UV, IR, ^1H NMR spectra were identical to those recorded on natural **2**. ^1H NMR (400 MHz, $CDCl_3$): δ1.85 (3H, *d*, J = 7.3 Hz, Me-C-11), 2.80 and 2.81 (3H each, *s*, Me-C-5, Me-C-5′), 4.70 (1H, *q*, J = 7.3 Hz, H-C-11), 7.11 (2H, *m*, H-C-6, H-C-6′), 7.21 (2H, *m*, H-C-8, H-C-8′), 7.41 (2H, *m*, H-C-7, H-C-7′), 11.86 and 12.58 (2H, exchangeable, OH-C-4, OH-C-4°).; ^{13}C NMR (100 MHz, $CDCl_3$): δ15.1 (*q*, Me-C-11), 23.5 and 23.6 (*q*, Me-C-5 and *q*, Me-C-5′), 26.9 (*d*, C-11), 106.5 and 106.8 (*s*, C-3 and *s*, C-3′), 114.9 (*d*, C-8 and *d*, C-8′), 116.0 (*s*, C-10 and *s*, C-10′), 128.2 (*d*, C-6 and *d*, C-6′), 131.5 and 131.7 (*d*, C-7 and *d*, C-7′), 138.5 (*s*, C-5 and *s*, C-5′), 153.4 and 153.6 (*s*, C-9 and *s*, C-9′), 167.2 and 167.6 (*s*, C-4 and *s*, C-4′), 168.1 and 168.8 (*s*, C-2 and *s*, C-2′).

Anhydro 11-*methylgerberinol* (**6**). Synthetic **2** on reaction with Ac_2O–H_2SO_4 (as given for natural **2**) gave **6**, mp 283° (98%) which was proved to be identical to the anhydro derivative preparted from natural **2** (IR, UV, ^1H NMR). ^{13}C NMR (100 MHz, $CDCl_3$): δ, 20.8 (*q*, Me-C-11), 22.6 (*q*, Me-C-5), 23.3 (*q*, Me-C-16), 24.5 (*d*, C-11), 100.9 (*s*, C-14), 108.4 (*s*, C-3), 112.1 (*s*, C-21), 115.3 (*d*, C-8), 115.6 (*d*, C-19), 128.1 (*d*, C-6), 128.5 (*d*, C-17), 131.7 (*d*, C-7), 132.6 (*d*, C-18), 136.3 (*s*, C-5), 141.4 (*s*, C-16), 153.7 (*s*, C-9), 154.5 (*s*, C-20), 156.2 (*s*, C-4), 157.5 (*s*, C-13), 160.4 (*s*, C-2), 178.7 (*s*, C-15).

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