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

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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, CH2 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 the Compositae plant *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 .... 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 13C NMR spectrum and the chemical shifts are fully consistent with the structure...
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–CHCl₃); C₂₂H₁₆O₅ [M⁺] 360.098; UV λmax 277, 300 nm; IR νmax 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, Sen-gupta and co-workers [3] observed that gerberinol (1), when treated with Ac₂O pyridine gives an anhydro compound (4) and this structure assignment was based on mechanism and spectral analysis (¹H NMR and ¹³C NMR). Structure 5 therefore looked likely for the anhydro derivative obtained by us from 11-methylgerberinol (2). However, ¹H NMR and ¹³C 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 ¹³C 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 H₂O. 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 λmax nm (log ε): 240 (4.31), 286 (4.30) and 320sh (4.10); IR νmax cm⁻¹: 3030, 1648, 1599, 1560, 1300, 1228, 1118 and 790; ¹H NMR (60 MHz, CDCl₃); δ2.80 (6H, s), 3.77 (2H, s), 6.9–7.5 (6H, two 1, 2, 3-trisubstituted benzene): EIMS (70 eV) m/z: 364.094 [M⁺] cal. for C₂₁H₁₆O₆ 364.095), base peak at m/z 134.

**Isolation of compound B (11-methyloerberinol, 2).** The dried and milled roots of D. kaki collected at Tokyo (2.0 kg) were extracted with CHCl₃ 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 λmax nm (log ε): 246 (4.12); 295 (4.41), 305 (4.34), 320 (4.12); IR νmax cm⁻¹: 1660, 1640, 1595, 1550, 1325, 1309, 1224, 745, ¹H NMR (60 MHz, CDCl₃); 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 Ac₂O–H₂SO₄.** To compound B (50 mg) in Ac₂O (4 ml) was added a few drops of conc. H₂SO₄ 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–CHCl₃ (46 mg); mp 283–284, UV λmax nm (log ε): 277 (4.09), 300 (4.33) and 333sh (3.82);
IR $\nu_{\text{max}}, \text{cm}^{-1}$: 1715, 1690, 1640, 1480, 1373, 1260, 1170, 1030, 795 and 780; $^1$H NMR (60 MHz, CDCl$_3$): $\delta$ 1.49 (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, $^1$H NMR spectra were identical to those recorded on natural 2. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.85 (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$_3$): $\delta$ 20.8 (q, Me-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).

Anhydro 11-methylgerberinol (6). Synthetic 2 on reaction with Ac$_2$O–H$_2$SO$_4$ (as given for natural 2) gave 6, mp 283° (98%) which was proved to be identical to the anhydro derivative prepared from natural 2 (IR, UV, $^1$H NMR). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 20.8 (q, Me-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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