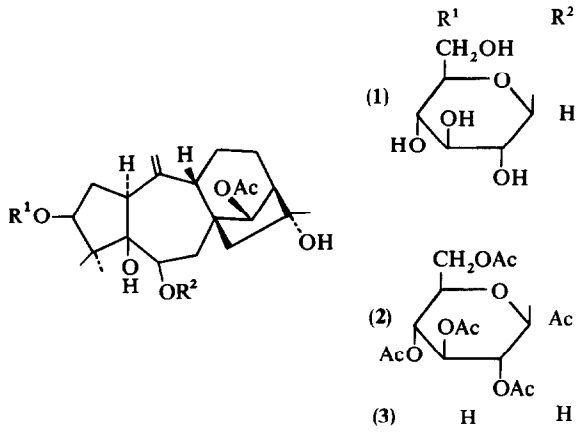

In general, carbinyl carbon (α-carbon) signals of aglycone alcohols are displaced by +7.0 ppm on glucosidation [3, 4]. The C-3 signal of the aglycone **3** was observed at δ 80.8, and the corresponding C-3 signal of the glucoside **1** was observed at δ 88.4. On the other hand, all other carbinyl carbon signals scarcely shifted on the glucosidation. Therefore, β-D-glucose must be attached at C-3 of **3**.

From these results, we can conclude that grayanoside A(**1**) is 5β,6β,16α-trihydroxy-14β-acetyloxy-3β-(β-D-glucopyranosyl)oxy-A-nor-B-homo-ent-kaur-10(20)-ene.

EXPERIMENTAL

Mps were uncorr. PMR spectra were measured at 100 MHz. ^{13}C-NMR spectra were measured at 15 MHz. Plants were collected at Hokkaido (northern island of Japan).

Extraction and isolation of **1**. Dry leaves and stems (4.3 kg) were extracted first with hot C_6H_6 and then with hot MeOH. The methanolic extracts were diluted with 3 litres of H_2O. The ppt was filtered off and then saturated lead subacetate soln was added to the filtrate. The resulting ppt was filtered and H_2S gas was bubbled into the filtrate. PbS was separated. The soln was concd *in vacuo* to 700 ml, and then extracted with $CHCl_3$, EtOAc and *n*-BuOH, successively. The *n*-BuOH extract was chromatographed on a column of activated charcoal. The MeOH–H_2O (60:40 to 65:35) eluate was concd to dryness and chromatographed on a Si gel column. The MeOH–$CHCl_3$ (15:85) eluate gave a syrup (2 g). The syrup (700 mg) was applied to a silanized Si gel column in MeOH–H_2O (20:80 to 40:60) to give **1** (100 mg).

Grayanoside A (**1**). Viscous syrup. $[\alpha]_D^{25}$ − 19.1 (MeOH c = 2.35), HPLC: JASCO FLC-150; 20% MeOH in H_2O 1.5 %/min gradient 0.75 ml/min, 50 cm × 2.1 mm φ, JASCO-DAC SV-02, UV detector operating at 204 nm (JASCO UVIDEC 100), retention time 5 min.

Pentaacetylgrayanoside A (**2**). Treatment of **1** with Ac_2O–Py for 15 hr at room temp. gave **2**; mp 196–199 (Et_2O); (Found: C 59.30; H 7.08. Calc. for $C_{38}H_{54}O_{16}$ C 59.52; H 7.10%).

Acid hydrolysis of **1**. A soln of **1** in dioxane (1 ml) and 5% H_2SO_4 (2 ml) was heated for 2.5 hr on a water bath. The mixture was cooled, diluted with H_2O (2 ml) and then extracted with EtOAc. The EtOAc extract was evapd *in vacuo* to give a complex mixture. The aq. layer was treated with Amberlite CG-4B-(OH^-) and evapd *in vacuo*. The sugar was converted to the TMSi derivative and identified as TMSi-D-glucose by GLC. The analysis of the TMSi-sugar was performed on a GLC equipped with FID and a stainless column, packed with 5% OV-1 (2 m × 3 mm) at 155°. Identification was made by comparison of the R_t with an authentic standard.

Enzymatic hydrolysis of **1**. To a soln of **1** (31 mg) dissolved in HOAc–NaOAc buffer (pH 4.1, 10 ml) crude naringinase 'SANKYO' (100 mg) was added and the reaction mixture was incubated for 16 hr at 40°. The product was extracted with EtOAc and the extract was purified by Si gel PLC (2 mm) with MeOH–$CHCl_3$ (1:9). The aglycone (**3**) was detected by I_2 vapor, and was eluted with MeOH–EtOAc (1:9), to yield crystals (6 mg). Compound **3** was identified as grayanotoxin IV by TLC, IR and mmp.

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NEW PHENOLIC DIGLYCERIDES FROM *AEGILOPS OVATA**

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Key Word Index—*Aegilops ovata*; Gramineae; phenolic fraction; scopoletin; *p*-coumaric acid; phenolic diglycerides; 1,3-diferulylglycerol; 1-ferulyl-3-*p*-coumarylglycerol; CMR spectroscopy.

Abstract—Two novel phenolic diglycerides have been isolated from *Aegilops ovata* together with scopoletin and *p*-coumaric acid. Spectroscopic evidence and a synthesis confirmed the proposed structures which are presented together with the CMR data for these diglycerides and related model compounds.

INTRODUCTION

The genus, *Aegilops* (Gramineae), is considered to be one of the ancestors of the cultivated wheat [1], *Triticum aestivum*. The genus *Triticum* has recently been examined for its flavonoid constituents [2]. During our studies on wild progenitors of wheat for the presence of naturally occurring germination inhibitors, an examination of the phenolic constituents of *Aegilops ovata* L. was undertaken [3, 4]. We now wish to report some other phenolics of *Aegilops ovata*.

*Part II in the series "*Constituents of the Gramineae*". For Part I see [3].

Table 1. ^{13}C NMR chemical shifts for the phenolic diglycerides and derivatives

Carbon	3c	5*	6†	3a	4‡
1	62.6	62.3		65.5	65.5
2	69.3	69.2		68.6	70.3
3	62.6	62.3		65.5	63 5
α′	145.1		144.2	146.0	144 9
β′	117.5		118.1	114.6	117 9
γ′	166.3		167.3	167.5	167.2
1′	133 2		133.4	126 8	133.4
2′	111.4		111.3	109 6	111.7
3′	151.5		151.5	148.4	151.5
4′	141.8		141.5	146.9	141.8
5′	123.4		123.3	114.9	122.9
6′	121.5		121.3	123.4	121.3
3′-OMe	56.0		55.9	56.0	56.0
2-OAc	169.2 20.6	170.5 20.8			
4′-OAc	168.7 20.6		168.9 20.7		

* δ(1-OAc) = 170.1 and 20.6 ppm.
† δ(CO_2Me) = 51.8 ppm.
‡ δ(CO_2Me) = 153 6 and 55.7 ppm.

RESULTS

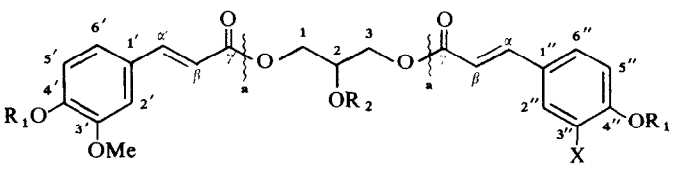
Chromatographic separation of the phenolic fraction from the fruits of *Aegilops ovata* gave four compounds: scopoletin **1** (30 mg), *p*-coumaric acid **2** (20 mg) and a mixture of two phenolic diglycerides **3a** and **3b**. Efforts to separate the two diglycerides were unsuccessful. From the PMR (90 MHz) spectrum, the presence of cinnamate derivatives linked to glycerol was indicated. After acetylating the mixture, triglycerides **3c** (50 mg) and **3d** (5 mg) were separated. From the PMR spectra, the signal, originally seen at δ 4.0 (m), was now shifted downfield to δ 5.4 (m) due to acetylation, and in both spectra there were now two acetyl signals (ratio 2:1) assigned to two aromatic-OAc and one aliphatic-OAc. By irradiating the glycerol protons in turn, the position of the C(2)-OAc was confirmed. The symmetrical nature of the ester linkages to this glycerol portion was also established by CMR. The carbon chemical shifts of the glycerol moiety of **3c** closely resemble those of other triglycerides [5], e.g. triacetin (**5**), while the acid residue is analogous to the acetate of methyl ferulate (**6**). Assignment of the signals of **6** was obtained using cinnamic acid as a model [5], and introducing appropriate substituent parameters [6, 7]. In the single-frequency off-resonance decoupled (sford) spectrum of **6**, the C(6′) signal shows a splitting by the *meta*-hydrogen not seen for C(5′) [6, 7]. This differentiation in **6** was thus applied to assignment of **3c** (see Table 1).

The MS of **3c** (M^+ 570) gave a base peak (*m/e* 177, $C_{10}H_9O_3$) suggesting the presence of a ferulic acid ester. This may have arisen from fragmentation pathway **a** after loss of two ketene moieties from the molecular ion. Of interest, however, was the absence of any peak indicating the loss of CH_3COOH (M^+-60) as might be expected if a secondary aliphatic acetate was present. For the triglyceride **3d**, (M^+ 540), the base peak was seen at *m/e* 147 ($C_9H_7O_2$) accompanied by a peak at *m/e* 177 (40%) suggesting the presence of both a coumaryl and ferulyl moiety in the same molecule.

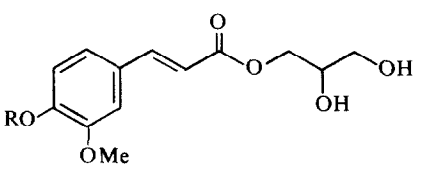
Unequivocal evidence for the proposed structures was provided by a synthesis of **3a**. Starting from ferulic acid, the carbomethoxyferulic acid chloride was reacted together with glycerol to give 1,3-dicarbomethoxyferulylglycerol (**3e**) together with 1-carbomethoxyferulylglycerol (**4**). 1,3-Diferulylglycerol (**3a**) was prepared by removal of the protecting group from **3e** using LiI in DMF. This diglyceride had an identical R_f on TLC to the naturally occurring compound, and upon acetylation gave a triglyceride whose analytical data agreed in all respects with that of the acetylated natural product (**3c**).

Since little information has been published on the CMR spectroscopy of partially acylated glycerol derivatives [8], we felt it would be of interest to examine the carbon spectra of this new diglyceride (**3a**) together with the monoglyceride (**4**). Assignment of the ferulyl moieties has been discussed (*vide supra*). The glycerol carbons could be identified by their multiplicities in the sford spectra and the assumed identity of the C(1) shifts in both compounds. A comparison between glycerol, where δ[C(1)] = 64.5 and δ[C(2)] = 70.3 ppm [9], monoglyceride (**4**), diglyceride (**3a**), and triglycerides (**3c**) and (**5**) shows that acylation causes, in every case, a small deshielding (0.6–2.0 ppm) of the hydroxy-carbon being esterified. A shielding of 1.7–2.9 ppm is seen for the adjoining carbon. Whilst these results are in good agreement with those obtained for simple alcohols [9], they could not have been predicted *a priori*.

Three phenolic triglycerides have recently been reported [10] from the Salicaceae. Together with the two new phenolic diglycerides reported here, these constituents form a new class of natural phenols. Other related cinnamate derivatives include chicoric acid [11] and cinnamyl flavonoids [12], -sugars [13] and -terpenes [14]. The fact that these phenolic diglycerides are found together with related phenolic compounds may indicate new pathways in cinnamic acid metabolism.



3a $R_1 = R_2 = H$, X = OMe
3b $R_1 = R_2 = X = H$
3c $R_1 = R_2$ = Ac, X = OMe
3d $R_1 = R_2$ = Ac, X = H
3e $R_1 = CO_2Me$, $R_2 = H$, X = OMe



4 R = CO_2Me

EXPERIMENTAL

CMR were recorded at 22.6 MHz on a Bruker WH-90 instrument operating in the Fourier transform mode. δ values are given in ppm using $CDCl_3$ solutions with TMS as internal standard. MS were determined under the supervision of Dr. Z. V. Zaretskii. All peaks are accurately mass measured and % values given in parentheses are based on values of base peak = 100%. Si gel 60 (E. Merk) was used for column chromatography, and TLC was carried out on Si gel chromatoplates (Riedel de Haen).

Details of the extraction procedure have been reported elsewhere [3]. The phenolic fraction (10 g) was chromatographed over Si gel, firstly with C_6H_6 (1 l.) followed by C_6H_6–$CHCl_3$ (1:1, 1 l.). This second fraction (1 g) was rechromatographed over Si gel and eluted with a C_6H_6–EtOAc gradient to give the 2 previously reported lignans [4], scopoletin (30 mg, mp 206–207°), a pale yellow oil (60 mg, **3a** and **3b**) and finally *p*-coumaric acid (20 mg, mp 210–212°).

Acetylation of the oil (**3a** + **3b**) (60 mg) with Ac_2O/Py. overnight at room temp. gave a mixture of 2 cpds separated by Si gel PLC (C_6H_6–EtOAc–MeOH, 11:10:1), yielding **3c** (50 mg) and **3d** (5 mg) having R_f 0.75 and 0.65 respectively.

3c Colourless oil; PMR 2.11 (3H, *s*, OAc), 2.31 (6H, *s*, 2× OAc), 3.86 (6H, *s*, 2× OMe), 4.30 (4H, *m*, which by double irradiation expts gave J = 12, 6, 4 Hz, 2× CH_2O), 5.40 (1H, *m*, —C<u>H</u>(OAc)—), 6.38 (2H, *d*, J = 16 Hz 2× ArCH=C<u>H</u>), 7.07 (6H, *br s*, 6× Ar—H), 7.66 (2H, *d*, J = 16 Hz, 2× Ar—C<u>H</u>=CH—). MS M^+ 570 ($C_{29}H_{30}O_{12}$, 2%), 528 ($C_{27}H_{28}O_{11}$, 16%), 486 ($C_{25}H_{26}O_{10}$, 12%), 219 ($C_{12}H_{11}O_4$, 5%), 177 ($C_{10}H_9O_3$, 100%).

3d Colourless oil, PMR, 2.12 (3H, *s*, OAc), 2.32 (6H, *s*, 2× OAc), 3.86 (3H, *s*, OMe), 4.38 (4H, *m*, which by double irradiation expts. gave J = 12, 6, 4 Hz, 2× C<u>H</u>$_2$O—), 5.40 (1H, *m*, —C<u>H</u>(OAc)—), 6.38 (2H, *d*, J = 16 Hz, 2× Ar—CH=C<u>H</u>), 7.07 (3H, *br s*, 3× Ar—H), 7.10 (2H, *d*, J = 8 Hz, H—3″—5″), 7.20 (2H, *d*, J = 8 Hz, H—2″—6″), 7.66 (2H, *d*, J = 16 Hz, 2× Ar—C<u>H</u>=CH—). MS M^+ 540 ($C_{28}H_{28}O_{11}$, 2%), 498 ($C_{26}H_{26}O_{10}$, 30%), 456 ($C_{24}H_{24}O_9$, 5%), 189 ($C_{11}H_9O_3$, 25%), 177 ($C_{10}H_9O_3$, 40%), 147 ($C_9H_7O_2$, 100%).

Synthesis of 1,3-*diferulylglycerol* (**3a**). Addition of methyl chloroformate to ferulic acid (3.5 g) in basic soln gave the carbomethoxyl derivative mp 186–7°. The acid chloride was obtained as a solid from $SOCl_2/C_6H_6$ and without further purification added to glycerol (0.5 g) in Py (50 ml) at 0° with stirring. The soln was kept at 0° for 1 h, left overnight at room temp. poured into H_2O (200 ml) and the oily lower layer separated. The aq. upper layer was acidified and extracted into Et_2O and combined with the oily organic layer, washed with dil. HCl, H_2O, then with a 5% $NaHCO_3$ soln to remove unreacted acid. The Et_2O layer, after washing (2× H_2O), drying, and removal of solvent, gave a yellow viscous oil. Passage of this oil through a Si gel column, eluting with C_6H_6–EtOAc (4:1), gave **3e** (900 mg) as a pure colourless oil, and further elution with EtOAc yielded **4** (250 mg).

Compound **3e** was dissolved in DMF (25 ml) and mixed with dry LiI (3 g) and NaOAc (200 mg) [15]. The mixture was refluxed under N_2 for 12 hr, cooled, poured into H_2O (100 ml), acidified with 5% HCl and extracted into Et_2O. The Et_2O layer was washed 3× with H_2O, dried, filtered and on removal of solvent a yellow oil (600 mg) remained. Purification by passage through a Si gel column and elution with C_6H_6–EtOAc (3:2) gave **3a** as an oil (520 mg) homogeneous on TLC (C_6H_6–EtOAc–MeOH, 55:50:5) R_f 0.5.

Compound **3a** (synthetic), colourless oil; UV λ_{max}^{MeOH} 230 nm: (log ε 3.7), 290 (3.8), 310 (4.1); M^+ 444 ($C_{23}H_{24}O_9$): PMR 3.82 (6H, *s*, 2× OMe), 4.0–4.3 (5H, *m*, —CH(OH) + 2× C<u>H</u>$_2$O—), 6.18 (2H, *d*, J = 16 Hz, 2× Ar—CH=C<u>H</u>), 6.85 (6H, *br s*, 6× Ar—H), 7.45 (2H, *d*, J = 16 Hz, 2× Ar—C<u>H</u>=CH).

Compound **4** colourless oil; M^+ 326 ($C_{15}H_{18}O_8$); PMR 3.78 (3H, *s*, —$COOCH_3$), 3.84 (3H, *s*, OMe), 4.0–4.3 (5H, *m*, —C<u>H</u>(OH) + 2× C<u>H</u>$_2$O—), 6.22 (1H, *d*, J = 16 Hz, Ar—CH=C<u>H</u>), 6.94 (3H, *br s*, 3× Ar—H), 7.42 (1H, *d*, J = 16 Hz, Ar—C<u>H</u>=CH).

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