TWO NEW LIGNAN GLUCOSIDES FROM ARUM ITALICUM

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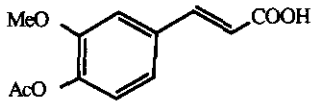
Abstract - Two new lignan glucosides, namely (+)-lariciresinol 4'-O-β-D-glucopyranoside and (+)-lariciresinol 9-O-β-D-glucopyranoside, besides ferulic acid, coniferyl alcohol 4-O-β-D-glucopyranoside, 4-coumaryl alcohol 4-O-β-D-glucopyranoside, (+)-pinoresinol-O-β-D-glucopyranoside, (+)-syringaresinol-O-β-D-glucopyranoside, (+)-lariciresinol and (+)-lariciresinol 4-O-β-D-glucopyranoside, have been isolated and characterized as peracetyl derivatives from Arum italicum.

In a search for bioactive compounds from aquatic plants we have recently studied Acorus gramineus[1] and Pistia stratiotes,[2] two Araceae containing phenylpropanes which inhibited the growth of several algal strains belonging to Cyanochloronta, Chrysophycophyta and Chlorophycophyta.



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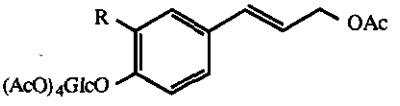
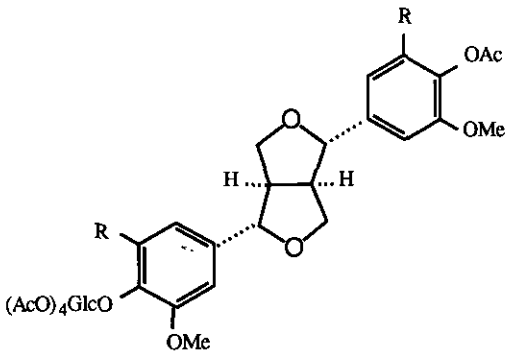
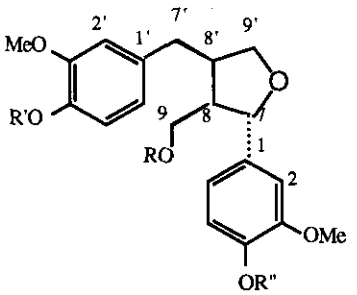


2 R = OMe **3** R = H



4 R = H **5** R = OMe



6 R = Ac R' = Ac R" = Ac
7 R = Ac R' = Ac R" = Glc(0Ac)$_4$
8 R = Ac R' = Glc(0Ac)$_4$ R" = Ac
9 R = Glc(0Ac)$_4$ R' = Ac R" = Ac

In pursuing our studies on these bioactive compounds we have now examined Arum italicum Miller, a species of the same family, and in this paper we report the isolation and the characterization of some phenylpropanes and lignans, two of them described for the first time.

The aqueous acetone extract of the rhizomes of A. italicum was distributed between ether and water and the aqueous layer was chromatographed on Amberlite XAD2. The residue from the methanolic fraction showed in the ir spectrum hydroxyl absorptions in the range 3600 - 3300 cm^{-1} and aromatic bands in the 1600 - 1500 cm^{-1} range. The ^{1}H-nmr spectrum in pyridine-d_5 showed no acetyl signals. It was subjected to chromatographic processes to give, after treatment with acetic anhydride in dry pyridine, the peracetyl derivatives of ferulic acid [**1**] , coniferyl alcohol 4-O-β-D-glucopyranoside [**2**],[3] 4-coumaryl alcohol 4-0-β-D-glucopyranoside [**3**],[4] (+)-pinoresinol-O-β-D-glucopyranoside [**4**],[4] (+)-syringaresinol-O-β-D-glucopyranoside [**5**],[5] (+)-lariciresinol [**6**],[6] (+)-lariciresinol 4-O-β-D-glucopyranoside [**7**][7] besides two new isomers of this latter, namely (+)-lariciresinol

Table 1. ^{13}C-nmr data of lariciresinol glucoside hexaacetates (**7** - **9**).

C	7*	7	8	9
1	136.6	136.5	139.0	141.9
2	113.3	108.6	110.3	109.6
3	150.7	150.9	150.9	150.7
4	144.7	144.8	138.8	138.8
5	108.8	120.2	122.8	122.6
6	120.4	118.0	117.9	117.6
7	82.9	83.0	82.3	82.4
8	56.2	49.0	49.0	50.6
9	72.8	62.8	62.5	69.9
1'	141.6	141.6	137.5	138.8
2'	117.8	113.5	116.3	112.6
3'	151.1	151.1	151.1	150.8
4'	139.1	139.1	145.6	138.0
5'	122.7	122.8	120.0	122.3
6'	120.6	120.5	120.5	120.4
7'	33.3	33.4	33.4	33.2
8'	55.9	42.3	42.2	42.4
9'	62.7	72.9	72.3	73.0
1-glc	100.9	100.8	101.5	100.8
2-glc	68.5	71.2	71.2	71.2
3-glc	71.3	72.7	72.6	72.7
4-glc	72.0	68.4	68.4	68.4
5-glc	72.7	72.0	72.0	72.0
6-glc	62.0	61.9	61.9	61.9
OMe	42.2	55.9	55.9	55.8
OMe	49.0	56.2	56.2	55.8

* Attributed by S. D. Jolad et al.[7]

4'-O-β-D-glucopyranoside [**8**] and (+)-lariciresinol 9-O-β-D-glucopyranoside [**9**]. All the known compounds were identified by comparison of their physical data with those previously reported.

In this context, however, a disagreement among the ^{13}C-nmr attributions in **7** reported by Jolad et al.[7] and those in analogous lignans[6,8,9] was observed so that an unambigous assignment based on 1D and 2D nmr experiments is now reported in Table 1.

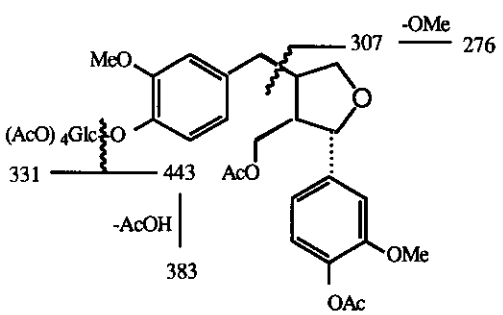
In the H-C one bond COSY the meta coupled proton at δ 6.94 (Table 2) was related to the carbon at δ 108.6 and the ortho and ortho - meta coupled protons at δ 6.99 and 6.86 gave correlations with the carbons at δ 120.2 and 118.0 respectively. In the H-C long range COSY the protons at δ 6.94 and 6.86 were correlated to the carbon at δ 144.8 while the proton at δ 6.99 gave cross peaks with the carbons at δ 150.9 and 136.5. This latter carbon was also related to the aliphatic doublet at δ 4.86 and this proton had in the H-H long range COSY correlations with the protons at δ 4.21 and 4.38, linked to the carbon at δ 62.8. Furthermore an interaction between the anomeric proton at δ 4.94 and the proton at δ 6.99 was evidenced in a nOe experiment.

Table 2. ^{1}H-nmr data of lariciresinol glucoside hexaacetates (**7** - **9**).

H	7	8	9
2	6.94 d (1.8)	6.75 d (2.6)	6.77 d (1.7)
5	6.99 d (8.1)	6.81 d (8.2)	6.99 d (8.2)
6	6.86 dd (1.8 8.1)	7.08 dd (2.6 8.2)	6.83 dd (1.7 8.2)
7	4.86 d (5.4)	4.80 d (5.4)	4.86 d (5.5)
8	2.56 m	2.55 m	2.48 m
9	4.38 dd (6.6 11.4) 4.21 dd (7.7 11.4)	4.39 dd (7.0 11.1) 4.22 dd (4.4 11.1)	4.11 dd (6.1 10.4) 3.75 overlapped
2'	6.69 d (2.1)	6.90 d (1.8)	6.96 d (2.1)
5'	7.04 d (7.9)	6.95 d (7.9)	6.94 d (7.7)
6'	6.67 dd (2.1 7.9)	6.74 dd (1.8 7.9)	6.75 dd (2.1 7.7)
7'	2.55 dd (12.0 12.7) 2.84 dd (4.6 12.7)	2.59 dd (10.3 13.0) 2.89 dd (4.7 13.0)	2.57 dd (10.4 13.0) 2.89 dd (4.7 13.0)
8'	2.70 m	2.73 m	2.71 m
9'	3.74 dd (6.9 8.6) 4.07 dd (6.5 8.6)	3.75 dd (6.8 8.7) 4.10 dd (6.2 8.7)	3.75 overlapped 4.04 dd (6.5 8.5)
OMe	3.81 s	3.82 s	3.83 s
OMe	3.84 s	3.83 s	3.85 s
1-glc	4.94 d (7.5)	4.95 d (7.5)	4.57 d (7.8)
2-glc	5.28 dd (7.5 7.5)	5.28 dd (7.5 7.5))	5.24 dd (9.3 9.3)
3-glc	5.28 dd (7.5 9.7)	5.28 dd (7.5 9.8)	5.16 dd (9.3 9.8)
4-glc	5.16 dd (9.7 9.7)	5.16 dd (9.8 9.8)	5.11 dd (9.7 9.8)
5-glc	3.76 m	3.76 m	3.73 overlapped
6-glc	4.16 dd (2.5 12.3) 4.28 dd (5.0 12.3)	4.17 dd (2.5 12.3) 4.29 dd (5.0 12.3)	4.15 dd (2.5 12.3) 4.29 dd (5.0 12.3)

On the other hand the protons of the second aromatic moiety at δ 6.67, 7.04 and 6.69 were related in the H-C one bond COSY to the carbons at δ 120.5, 122.8 and 113.5 respectively. Furthermore the protons at δ 6.67 and 6.69 gave cross peaks with the carbon at δ 139.1 in the H-C long range COSY and had correlations with the double doublets at δ 2.55 and 2.84 in the H-H long range COSY. Finally these protons were related to the double doublets at δ 3.74 and 4.07, linked to the carbon at δ 72.9.

Compound [**8**] had $[\alpha]_D$ - 78° and the same molecular formula $C_{38}H_{46}O_{17}$ of **7** according to a peak at m/z 774 in the positive FAB mass spectrum, the presence of 38 carbons in an inverse gated decoupled ^{13}C-nmr spectrum and the elemental analysis. The aliphatic region of the ^{1}H-nmr spectrum (Table 2), showed signals whose chemical shifts and couplings were comparable to those of **7** thus suggesting an identical central grouping. In the aromatic region were present six aromatic protons which were assigned, on the basis of decoupling experiments, to two-1,2,4 trisubstituted benzenes. The chemical shifts of these protons and those of the corresponding carbons as well as the nOe effects of the methoxyl groups only on the meta coupled protons justified a substitution pattern identical to that of lariciresinol in both the rings and, accordingly, the sequence of alkaline and enzymatic hydrolisis of **8** gave an aglycone which, after acetylation, was identical to (+)-lariciresinol triacetate [**6**] $[\alpha]_D$ +8°. On these bases it was supposed the structure (+)-lariciresinol 4'-O-β-glucopyranoside hexaacetate. The differences in the ^{1}H- and ^{13}C-nmr shieldings of the aromatic signals in **7** and **8** justified the linkage of the glucose moiety at the C-4' position instead of C-4. Accordingly in the H-H long range COSY the H-7' protons at δ 2.59 and 2.89 were correlated to the aromatic H-6' at δ 6.74 and in a nOe experiment the 1H-glc gave interaction with the H-5' proton at δ 6,95, coupled to the above H-6'; furthermore in the FABms spectrum a peak at m/z 307 (Scheme 1), due to the loss of the glucosylated benzylic residue, was present.



Scheme 1. Major fragment ions in the FAB mass spectrum of compound (**8**).

Compound [**9**], $[\alpha]_D \pm 0°$, exhibited in the FAB mass spectrum a peak at m/z 774 as the previous lignans and by hydrolysis and acetylation gave (+)-lariciresinol triacetate [**6**]. The analysis of the ^{13}C-nmr spectrum showed that the C-4' and C-4 carbons had chemical shifts comparable with those of **7** and **8** respectively, thus justifying the presence of two acetyl residues at these positions, while the C-9 carbon was downfield shifted of about 7

ppm. This shift, attruibutable to a glycosilation effect, justified the structure (+)-laricirersinol 9-O-β-D-glucopyranoside hexaacetate.

EXPERIMENTAL

General experimental procedures. Nmr spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C on a Bruker AC 400 spectrometer in $CDCl_3$. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. FAB mass spectra were obtained with a VG ZAB 2SE apparatus and ei mass spectra were obtained with a Kratos MS 80 apparatus.

Plant material. The whole plants of Arum italicum Miller were collected from Agerola (Naples) in the month of September. Identification was carried out by prof G. Aliotta and a voucher specimen is available in the herbarium of the Dipartimento di Biologia Vegetale of the University of Naples.

Extraction and isolation. The dried rhizomes (19 Kg) of A. italicum were extracted with aqueous acetone (1 : 1, 10 l) at 50° C for 2 weeks. After percolation the extract was evaporated in vacuo and the residue (266 g) was distributed between ether (2 l) and water (2 l). The aqueous layer was chromatographed on Amberlite XAD2 and the methanolic fraction (1.5 g) was chromatographed on silica gel. Elution with $CHCl_3$ - MeOH (19 : 1) gave a crude product (15 mg) which, acetylated with Ac_2O (1 μl) in dry pyridine (1 ml) overnight at room temperature and purified by preparative tlc ($CHCl_3$ - AcOEt 47 : 3) was identified as (+)-lariciresinol triacetate [**6**]: oily, (4 mg); $[\alpha]_D$ +8° (c 1.0 in $CHCl_3$); eims: m/z (%) 486 (25), 442 (20), 402 (21), 384 (33), 367 (35), 342 (35), 325 (100), 219 (28); ^1H-nmr: δ 7.01 (d, 8.1, H-5), 6.97 (d, 1.9, H-2'), 6.95 (d, 7.9, H-5'), 6.88 (dd, 2.1 and 8.1, H-6), 6.78 (d, 2.1, H-2), 6.75 (dd, 1.9 and 7.9, H-6'), 4.88 (d, 5.5, H-7), 4.38 (dd, 6.6 and 11.2, H-9), 4.23 (dd, 7.8 and 11.2, H-9), 4.08 (dd, 6.5 and 8.7, H-9'), 3.74 (dd, 6.9 and 8.7, H-9'), 2.89 (dd, 5.0 and 12.9, H-7'), 2.74 (m, H-8'), 2.59 (m, H-7' and H-8), 3.85 and 3.83 (ss, OMe); ^{13}C-nmr: δ 138.6 (C-1), 109.7 (C-2), 150.8 (C-3), 141.3 (C-4), 122.5 (C-5), 117.6 (C-6), 82.9 (C-7), 49.1 (C-8), 62.7 (C-9), 138.3 (C-1'), 112.5 (C-2'), 150.7 (C-3'), 138.8 (C-4'), 122.6 (C-5'), 120.2 (C-6'), 33.3 (C-7'), 42.3 (C-8'), 72.9 (C-9'), 55.8 (2 x OMe).

The fraction eluted with $CHCl_3$ - MeOH (9 : 1; 80 mg) was chromatographed on preparative tlc ($CHCl_3$ - MeOH 3 : 1) to give after acetylation **1** (4 mg), purified by preparative tlc ($CHCl_3$ - AcOEt 4 : 1), and a mixture of **4** (24 mg) and **5** (8 mg) which was resolved by preparative tlc ($CHCl_3$ - AcOEt 7 : 3).

The fractions eluted with $CHCl_3$ - MeOH -H_2O (14 : 6 : 1; 150 mg) were acetylated with Ac_2O (0.5 ml) in dry pyridine (3 ml) overnight at room temperature and chromatographed on silica gel eluting with benzene - AcOEt (3 : 1). The less polar fractions after purification by preparative tlc ($CHCl_3$ - AcOEt 4 : 1) gave (+)-lariciresinol 9-O-β-D-glucopyranoside hexaacetate [**9**] as an amorphous powder (10 mg): FAB ms m/z 774, 595, 443, 331; Anal. Calcd. for $C_{38}H_{46}O_{17}$: C, 58.91; H, 5.94. Found: C, 58.83; H, 5.84. The most polar fractions after preparative tlc (benzene - AcOEt 3:1) gave **2** (8 mg), **3** (40 mg) and a mixture of **7** and **8** (41 mg) which was resolved by HPLC (LiChrosorb Si-60 (Merck), hexane - acetone 7 : 3). (+)-Lariciresinol 4-O-β-D-glucopyranoside hexaacetate [**7**] (25 mg) (amorphous powder) had: $[\alpha]_D$ - 8.2° (c 1.1 in $CHCl_3$); FAB ms: m/z 797, 774, 384, 331. (+)-Lariciresinol 4'-O-β-D-glucopyranoside hexaacetate [**8**] (amorphous powder) (12 mg)

$[\alpha]_D$ - 78° (c 1.3 in $CHCl_3$); FAB ms m/z 797, 774; Anal. Calcd. for $C_{38}H_{46}O_{17}$: C, 58.91; H, 5.94. Found: C, 59.01; H, 5.87.

(+)-Lariciresinol triacetate [6] from **8** and **9**. Pure glucoside (10 mg, 0.01 mmol) was treated with 5% methanolic KOH (3 ml, 2.6 mmol) for 3 h at room temperature and the reaction mixture, neutralized with Amberlite IR-120, was filtered and evaporated. The residue was hydrolized with β-glucosidase (10 mg, Sigma Chemical Company) in acetate buffer (O.1 M AcOH - 0.1 M AcONa 1:2, 5ml) for 3 days at 37°C. The reaction mixture was extracted with ethyl acetate to give crude lariciresinol (6 mg) which was treated with excess acetic anhydride in dry pyridine (1 ml) overnight at room temperature. Preparative tlc of the reaction product (silica gel, $CHCl_3$ - AcOEt 47:3) gave pure **6** (3 mg, 0.006 mmol, 60% yield). The aqueous layer was lyophilized and the pc chromatography of the residue (BuOH - AcOH - H_2O 4 : 1 : 2) gave D-glucose (Rf 0.27).

ACKNOWLEDGMENTS

This work was supported by Consiglio Nazionale delle Ricerche (CNR) Rome in the frame of the "Progetto Finalizzato Chimica Fine".

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Received, 29th March, 1993