DIHYDROBENZOFURAN NEOLIGNANS FROM ARUM ITALICUM

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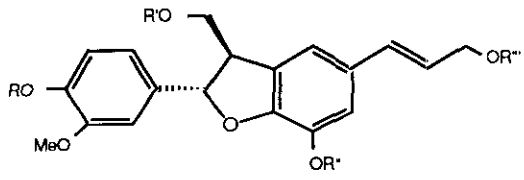
Abstract - A mixture of two new enantiomeric dihydrobenzofuran neolignans has been isolated from Arum italicum. The structures have been determined on the basis of the physical data of the compounds.

In a search for bioactive compounds with cytotoxic activity some new lignans[1] and 8-O-3' and 8-O-4' neolignans[2] were recently isolated from Arum italicum Miller and in pursuing such a study we now report the isolation of some dihydrobenzofuran neolignans.

The methanolic extract of the plant was subjected to repeated chromatographic processes to give a mixture of two new enantiomeric dihydrobenzofuran neolignans (**1a**) and (**2a**) besides a mixture of the enantiomeric dehydrodiconiferyl alcohols (**1c**) and (**2c**)[3] and the corresponding 4-O-β-D-glucopyranosides (**1d**) and (**2d**)[4] and 9-O-β-D-glucopyranosides (**1e**) and (**2e**).[5]



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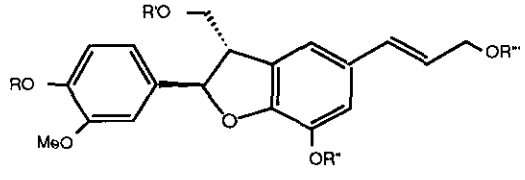

2

a R = R' = R" = R"' = H
b R = R' = R" = R"' = Ac
c R = R' = R"' = H R" = Me
d R = glc R' = R"' = H R" = Me
e R = R"' = H R' = glc R" = Me
f R = R' = R"' = H R" = glc

The already known glucosylated neolignans (**1d**, **2d**, **1e** and **2e**) were identified on the basis of their ^{1}H- and ^{13}C-nmr data. The cd spectra of **1d** and **2d**, as well as **1e** and **2e**, showed an intense transition at 285 and 270 nm, but with opposite signs and a comparison with the data previously reported for dihydrobenzofuran neolignans[6] allowed to assign the absolute configurations 7S,8R to the aglycone in **1d** and **1e** and 7R,8S to the aglycone in **2d** and **2e**. Enzymatic hydrolisis with β-glucosidase of **1e** and **2e** gave the pure enantiomers **1c** and **2c** and a comparison of their cd curves with that of the natural dehydrodiconiferyl alcohol showed that this latter consisted of an enantiomeric mixture with the predominance of **2c**.

The mixture of the new neolignans (**1a**) and (**2a**), $[\alpha]_D$ - 4°, showed in its HR mass spectrum a molecular ion at m/z 344.1248 for a molecular formula $C_{19}H_{20}O_6$ and in its ir spectrum absorptions of hydroxyl functions at 3335 cm^{-1} and aromatic rings at 1600 and 1508 cm^{-1}. Acetylation with acetic anhydride in dry pyridine gave a product showing in the ^{1}H-nmr spectrum ($CDCl_3$) two acetate signals at δ 2.09 and 2.10 and two acetate signals at δ 2.32 according to the presence of two alcoholic and two phenolic functions. The ^{1}H-nmr spectrum (CD_3OD) of **1a** and **2a** showed in the aromatic region three protons at δ 6.98 (d, J = 2.1 Hz), 6.86 (dd, J = 2.1 and 8.0 Hz) and 6.75 (d, J = 8.0 Hz) which were attributed, on the basis of decoupling experiments, to an 1,3,4 trisubstituted benzene and two protons as a singlet at δ 6.72. In the aliphatic region beside a methoxyl methyl at δ 3.81, were present a doublet at δ 6.53 (J = 16.0 Hz) coupled to a double triplet at δ 6.23 (J = 6.2 and 16.0 Hz) which, in turn, was coupled to a doublet for two protons at δ 4.18 (J = 6.2 Hz). Beside these systems attributable to a propenolic chain was present a doublet at δ 5.49 (J = 6.0 Hz) coupled to a multiplet at δ 3.44 which was coupled to AB double doublets at δ 3.78 (J = 7.9 and 11.0 Hz) and 3.80 (J = 6.4 and 11.0 Hz). The nOe interaction between the methoxyl signal and the doublet at δ 6.75 and the correlations in the H-H long range COSY of the methine at δ 5.49 with the signals at δ 6.75 and 6.86 allowed the location of the methoxyl at C-3 and the hydroxyl at C-4.

In the H-C one bond COSY the aromatic protons at δ 6.72 were related to the carbons at δ 116.2 and 118.0. The correlations of these carbons with the olefinic proton at δ 6.53 and those of the aromatic protons at δ 6.72 with the carbon at δ 149.1 located the side chain at C-1' and the hydroxyl function at C-5' univocally. A comparison of the cd spectrum with that of dehydrodiconiferyl alcohol finally indicated the presence of both the enantiomers (**1a**) and (**2a**). Such a mixture might be generate through a nonenantiospecific oxidative coupling of coniferyl alcohol with 3,4-dihydroxy cinnamyl alcohol.

1a and **2a** have not been previously reported as natural products, however, H. Wang et al. recently described[7] the isolation from Stauntonia chinensis of Yemuoside YM1 (**1f**), the 3'-glucoside of the (7S,8R) enantiomer (**1a**).

EXPERIMENTAL

Nmr spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Bruker AC 400 spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Ei mass spectra were obtained with a Kratos MS 80 apparatus. The cd spectra were recorded in EtOH solutions on a Jasco J-5OO apparatus. 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.

Isolation of neolignans. The dried rhizomes (19 Kg) of A. italicum were extracted with aqueous acetone (1 : 1) after percolation for 2 weeks at room temperature. The extract was evaporated in vacuo and the residue (266 g) was distributed between ethyl ether and water. The aqueous layer (120 g) was chromatographed on Amberlite XA D2 and the methanolic fraction (11.5 g) was chromatographed on silica gel eluting with mixture $CHCl_3$ - MeOH. The fractions eluted with a mixture 23 : 2, subjected to flash chromatography. $CHCl_3$ - MeOH (19:1), gave the mixture of dydrodiconiferyl alcohol (**1c** and **2c**) (19 mg): cd (c = 0.008) [q] (nm): + 2,420 (270), + 3,280

(285). The fractions eluted with $CHCl_3$ - MeOH (22:3) gave the crude mixture of **1a** and **2a** which was purified by chromatography with $CHCl_3$ - MeOH - H_2O (35:8:2): (20 mg); $[\alpha]_D$ - 4° (c = 1.2 in EtOH); cd (c = 0.010) [θ] (nm): + 2,940 (270), + 4,020 (285); ^{1}H-nmr δ (CD_3OD): 6.98 (1H, d, J = 2.1 Hz, H-2), 6.75 (1H, d, J = 8.0 Hz, H-5), 6.86 (1H, dd, J = 2.1 and 8.0 Hz, H-6), 5.49 (1H, d, J = 6.0 Hz, H-7), 3.46 (1H, m, H-8), 3.78 (1H, dd, J = 7.9 and 11.0 Hz, H-9), 3.80 (1H, dd, J = 6.6 and 11.0 Hz, H-9), 6.72 (2H, s, H-2' and H-6'), 6.53 (1H, d, J = 16.0 Hz, H-7'), 6.23 (1H, dt, J = 6.2 and 16.0 Hz, H-8'), 4.18 (2H, d, J = 6.2, H-9'), 3.81 (3H, s, OMe); ^{13}C-nmr δ (CD_3OD): 134.1 (C-1), 110.6 (C-2), 149.1 (C-3), 147.8 (C-4), 116.3 (C-5), 120.0 (C-6), 89.8 (C-7), 54.6 (C-8), 65.0 (C-9), 131.2 (C-1'), 118.1 (C-2'), 131.8 (C-3'), 149.3 (C-4'), 142.7 (C-5'), 116.4 (C-6'), 132.3 (C-7'), 127.2 (C-8'), 63.8 (C-9'), 56.4 (OMe). Ac_2O - pyridine gave the mixture of tetraacetates (**1b**) and (**2b**): ^{1}H-nmr δ ($CDCl_3$): 7.04 (1H, d, J = 1.5 Hz, H-2), 7.02 (1H, d, J = 8.1 Hz, H-5), 6.89 (1H, dd, J = 1.5 and 8.1 Hz, H-6), 5.59 (1H, d, J = 5.9 Hz, H-7), 3.72 (1H, m, H-8), 4.32 (1H, dd, J = 8.5 and 11.2 Hz, H-9), 4.46 (1H, dd, J = 6.0 and 11.2 Hz, H-9), 7.03 (1H, s, H-2'), 7.12 (1H, s, H-6'), 6.57 (1H, d, J = 15.8 Hz, H-7'), 6.16 (1H, dt, J = 6.6 and 15.8 Hz, H-8'), 4.70 (2H, d, J = 6.3, H-9'), 3.83 (3H, s, OMe); ^{13}C-nmr δ ($CDCl_3$): 139.6 (C-1), 109.5 (C-2), 151.1 (C-3), 139.2 (C-4), 122.9 (C-5), 117.9 (C-6), 88.1 (C-7), 51.0 (C-8), 65.4 (C-9), 127.5 (C-1'), 121.4 (C-2'), 133.1 (C-3'), 151.0 (C-4'), 135.7 (C-5'), 120.5 (C-6'), 131.4 (C-7'), 121.5 (C-8'), 65.4 (C-9'), 56.0 (OMe).

The fractions eluted with $CHCl_3$ - MeOH (17 : 3) consisted of a mixture of **1d** and **2d**. Hplc (ODS, MeOH - H_2O 1:3) gave pure **1d** {15 mg; cd (c = 0.009) [q] (nm): - 10,640 (270), - 13,790 (285)} and **2d** {35 mg; cd (c = 0.009) [q] (nm): + 12,200 (270), + 15,740 (285)}.

The fractions eluted with $CHCl_3$ - MeOH (7 : 3) were resolved by hplc (ODS, H_2O - THF 7:13) to give **1e** {20 mg), cd (c = 0.008) [q] (nm): - 13,100 (270), - 16,090 (285)} and **2e** {48 mg, cd (c = 0.008) [q] (nm): +12,200 (270), + 16,400 (285)}.

Enzymatic hydrolyis s of **1e** and **2e**. A pure sample of **1e** (10 mg) was hydrolyzed with b-glucosidase (5 mg, Sigma Chemical Co.) in acetate buffer (0.1 N HOAc - 0.1 M NaOAc 1:2, 5 ml, pH 5.0) for 1 day at 37 °C. The reaction mixture was extracted with Et_2O and the residue was chromatographed on silica gel ($CHCl_3$ - MeOH 20:1) to give pure **1c** (4 mg) cd (c 0.008) [q] (nm): -11,380 (270), - 14,300 (285). In the same conditions **2e** (10 mg) gave **2c** (4 mg) cd (c 0.008) [q] (nm): + 9,780 (270), + 12,700 (285).

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