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TWO POLYHYDROXYSTILBENES FROM STEMS OF *PHOENIX DACTYLIFERA*

MA. ISABEL FERNÁNDEZ, JOSÉ R. PEDRO and ELISEO SEOANE

Department of Organic Chemistry, Faculty of Chemistry, University of Valencia, Burjasot, Valencia, Spain

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Key Word Index—*Phoenix dactylifera*; Palmae; stem; resveratrol; *cis*- and *trans*-polyhydroxystilbenes.

Abstract—From stems of *Phoenix dactylifera* *trans*-3,5,3′,5′-tetrahydroxy-4-methoxystilbene has been isolated as a major component; *cis*-3,5,3′,5′-tetrahydroxy-4-methoxystilbene and *trans*-3,5,4′-trihydroxystilbene were isolated as minor components. Other metabolites from the biogenetic route to these stilbenes were also characterized.

INTRODUCTION

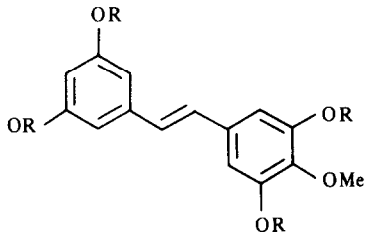
In a recent short paper [1] we described, together with the literature on date and oil seeds of *Phoenix dactylifera*, the isolation and characterization of 3-hydroxyphytosterols, 3,6-diketophytosterols and 3-keto-6-hydroxyphytosterols from hexane extracts of their large side stems. We now wish to report on polyhydroxystilbenes isolated from alcoholic extracts of the same plant parts.

RESULTS AND DISCUSSION

The alcohol extract was separated into acidic and neutral fractions. From the neutral fraction chromatographed on silica gel hexane–ether eluted successively crystalline compounds **1***, **2a** and **3a**.

Compound **1** was identified as *trans*-3,5,4′-trihydroxystilbene (resveratrol) [2] on the basis of its spectroscopic properties and mp 260–261°.

Compounds **2a** and **3a** are not described in the literature and have been identified as *cis*- and *trans*-3,5,3′,5′-tetrahydroxy-4-methoxystilbene, respectively, on the evidence of their properties and derivatives. **3a**, by molecular isotopic ions, gave a molecular formula $C_{15}H_{14}O_5$. Its IR spectrum showed bands for hydroxyl groups (3250 cm^{-1}), a *trans*-double bond (980 cm^{-1}) and *m*-trisubstituted aromatic rings (1620, 1595, 1525, 1435, 835 and 670 cm^{-1}). ^1H NMR signals confirmed the presence of the double bond (δ6.85), phenolic groups and *m*-trisubstituted aromatic rings. Its UV spectrum in ethanol showed maxima (log ε) at 305 (3.59) and 315 (3.56) nm.



R

3a H

3b Ac

3c Me

Compound **3a** treated with acetic anhydride and pyridine gave a derivative identified as 3,5,3′,5′-tetraacetoxy-4-methoxystilbene (**3b**). A molecular formula of $C_{23}H_{22}O_9$ was inferred from quantitative elemental analysis and the $[M]^+$ peak of the mass spectrum of **3a**. In its mass spectrum the fragment ions *m/z* 400 (22) $[M-42]^+$, 358 (51) $[M-2\times42]^+$, 316 (36) $[M-3\times42]^+$, 274 (25) $[M-4\times42]^+$ showed the derivative to be a tetraacetyl compound, which was confirmed in the ^1H NMR spectrum by four singlets at δ2.28, 2.30, 2.32 and 2.34. Other NMR signals established the presence of a double bond (δ7.05), a methoxyl group (δ3.85) and *m*-trisubstituted rings. Moreover, the structure of the tetraacetylated compound was proved by degradation; potassium permanganate oxidation with phase transfer gave 3,5-diacetoxybenzaldehyde and 4-methoxy-3,5-diacetoxybenzaldehyde

When 3,5,3′,5′-tetraacetoxy-4-methoxystilbene was hydrogenated it gave 3,5,3′,5′-tetraacetoxy-4-methoxybibenzyl, characterized by its spectroscopic properties. The disappearance of the double bond absorption was seen in the IR spectrum, and in the NMR spectrum by a new singlet at δ2.90 for $2\times CH_2Ar$. Its mass spectrum showed after four consecutive ketene losses a benzylic cleavage [3] giving peaks at *m/z* 153 and 123.

Finally the methylation of **3a** with diazomethane gave 3,4,5,3′,5′-pentamethoxystilbene (**3c**), mp 134–135°, previously described by Drews and Fletcher [4].

Compound **2a** was identified as *cis*-3,5,3′,5′-tetrahydroxy-4-methoxystilbene. Its spectral properties were the same as those of its stereoisomer, **3a**, with the exception of properties related to the double bond, which appeared at 680 cm^{-1} in the IR spectrum and at δ6.55 in the ^1H NMR spectrum (instead of 980 cm^{-1} and δ6.86,

*Compounds **1**, **2a** and **2b** are well known and are not illustrated.

respectively). The UV maxima were also different: 225 (4.22) and 294 (3.67) nm. Final identification was carried out by synthesis from the irradiation of the *trans*-stereoisomer. Natural stilbenes occur almost exclusively in the more stable *trans*-form; some cases of naturally occurring *cis*-stilbenes have been reported [4, 5].

p-Hydroxybenzoic acid and 3,5-dihydroxy-4-methoxybenzoic acid were isolated from the acidic fraction of the alcoholic extract. The isolation of these two acids, metabolites of the biogenetic route from shikimic acid, together with related stilbenes, is in accordance with the hypothesis that one ring of stilbenes comes from shikimic acid, the other one (3,5-dihydroxy ring) being synthesized via the acetate–malonate biogenetic route [6, 7].

Stilbenes are considered as antimicrobiological agents responsible for the durability of heartwood of various tree species [8] and they have been isolated from several higher plants [9].

EXPERIMENTAL

General. Mps are uncorr. IR spectra were recorded as liquid films or as KBr discs. ^{1}H NMR spectra were determined with TMS as int. standard.

Isolation of stilbenes. P. dactylifera identified by Prof. Mansanet (University of Valencia) was collected from the Municipal Park at Elche. Stems, air-dried and powdered (4.1 kg), were successively extracted with hexane, $CHCl_3$ and EtOH. The EtOH extract (260 g) dissolved in H_2O–EtOH (2:1) was continuously re-extracted with Et_2O. The Et_2O extract was separated by treatment with $NaHCO_3$ soln into a neutral (13.3 g) and an acidic fraction (8.6 g). The neutral fraction was chromatographed on silica gel (305 g) and eluted with hexane containing increasing amounts of Et_2O. Hexane–Et_2O (11:14) eluted successively a crystalline compound, **1** (40 mg) and an oily compound, **2a** (92 mg). Hexane–Et_2O (8:17) subsequently eluted crystalline compound **3a** (1205 mg).

Cis-3,5,3′,5′-*tetrahydroxy*-4-*methoxystilbene* (**2a**). Oil, giving one GC peak after trimethylsilylation; IR ν_{max} cm^{-1}: 3370 (OH), 3005, 2850, 1590, 1520, 1440 (aromatic), 1155 (C–O), 860, 845 (substituted ring), 680 (*cis*-CH=CH); UV λ_{max}^{EtOH} nm (log ε): 225 (4.22) and 294 (3.67), with bathochromic shift in NaOEt to 240 and 305; ^{1}H NMR [$(CD_3)_2CO$] (60 MHz): δ3.85 (*s*, 3H, OMe), 6.45 (*m*, 5H aromatic), 6.55 (*s*, 2H, CH=CH), 8.10 and 8.37 (2 *s*, 4H, 4OH).

Cis-3,5,3′,5′-*tetraacetoxy*-4-*methoxystilbene* (**2b**). **2a** (40 mg, 0.146 mmol) in pyridine was treated with an excess of Ac_2O (0.2 ml) overnight. The reaction product was extracted with Et_2O and purified by CC of silica gel with hexane–Et_2O (7:3) to give *cis*-3,5,3′,5′-tetraacetoxy-4-methoxystilbene (57 mg) as an oil; IR ν_{max} cm^{-1}: 3040, 2970, 2850, 1770 (CO of phenolic acetate), 1610, 1580, 1500, 1430 (aromatic), 1370 (Me), 1190, 895 and 660 (substituted ring) and 690 (*cis*-CH=CH); ^{1}H NMR ($CDCl_3$) (60 MHz): δ2.26 and 2.30 (2*s*, 12H, 4 × AcO), 3.83 (*s*, 3H, OMe), 6.57 (*s*, 2H, CH=CH), 6.9 (dist. *t*, 1H, J = 2 Hz, H-4′), 7.04 (*s*, 2H, H-2 and H-6), 7.06 (*d*, 2H, J = 2 Hz, H-2′ and H-6′); MS *m/z* (rel. int.): 442 [M]$^+$ (24), 400 [M − 42]$^+$ (40), 358 [M − 2 × 42]$^+$ (86), 316 [M − 3 × 42]$^+$ (57), 274 [M − 4 × 42]$^+$ (40).

Trans-3,5,3′,5′-*tetrahydroxy*-4-*methoxystilbene* (**3a**). Crystalline, mp 165–166° (from MeOH–$CHCl_3$); IR ν_{max} cm^{-1}: 3250, (OH), 3005, 2840, 1620, 1595, 1525, 1435, (aromatic), 1160, 980 (*trans*-CH=CH), 835 and 670 (substituted ring); UV λ_{max}^{EtOH} nm (log ε): 305 (3.59) and 315 (3.56), with bathochromic shift to 310 and 320 in NaOEt; ^{1}H NMR [$(CD_3)_2CO$] (60 MHz): δ3.85 (*s*, 3H, OMe), 6.30 (*t*, 1H, J = 2 Hz, H-4′), 6.55 (*d*, 2H, J = 2 Hz, H-2′ and H-6′), 6.62 (*s*, 2H, H-2 and H-6), 6.85 (*s*, 2H, CH=CH), 7.95 (*br s*, 2H, 2 × OH), 8.22 (*br s*, 2H, 2 × OH); MS *m/z* (rel. int.): 274 [M]$^+$ (100), 241 (47), 213 (71), 185 (22).

Trans-3,5,3′,5′-tetraacetoxy-4-*methoxystilbene* (**3b**). **3a** (80 mg, 2.92 mmol) treated in pyridine (10 ml) with excess of Ac_2O (4 ml) overnight gave after working up *trans*-3,5,3′,5′-tetraacetoxy-4-methoxystilbene (1.2 g), mp 110–111° (from MeOH); (Found: C, 62.21; H, 4.93. $C_{23}H_{22}O_9$ requires: C, 62.44; H, 4.97%); IR ν_{max} cm^{-1}: 3015, 2975, 1760 and 1775 (CO of phenolic acetate), 1610, 1580, 1500, 1440 (aromatic), 1370 (Me), 1190, 950 (*trans*-CH=CH), 890 and 665 (substituted ring); ^{1}H NMR ($CDCl_3$) (60 MHz): δ2.28, 2.30, 2.32 and 2.34 (4*s*, 12H, 4 × AcO), 3.85 (*s*, 3H, OMe), 7.08 (2H, CH=CH), 7.25 (*m*, 5H aromatic); MS *m/z* (rel. int.) 442 [M]$^+$ (17), 400 [M − 42]$^+$ (22), 358 [M − 2 × 42]$^+$ (51), 316 [M − 3 × 42]$^+$ (36), 274 [M − 4 × 42]$^+$ (25).

Degradation of trans-3,5,3′,5′-*tetraacetoxy*-4-*methoxystilbene.* 150 mg **3b** (0.34 mmol) in C_6H_6 (2 ml) was treated with $KMnO_4$ (150 mg, 0.95 mmol) and *n*-butylammonium bromide (15.3 mg) in H_2O (1.5 ml) with stirring for 2.5 hr at room temp. After elimination of excess $KMnO_4$ with $NaHSO_3$ and C_6H_6 extraction, TLC on silica gel separated unreacted starting product, 3,5-diacetoxybenzaldehyde and 4-methoxy-3,5-diacetoxybenzaldehyde.

3,5-*Diacetoxybenzaldehyde*. IR ν_{max} cm^{-1}: 2720 and 1700 (CHO); positive test with 2,4-DNPH; ^{1}H NMR ($CDCl_3$) (60 MHz): δ2.34 (*s*, 6H, 2 × AcO), 7.25 (*t*, 1H, J = 2 Hz, H-4), 7.55 (*d*, 2H, J = 2 Hz, H-2 and H-6), 9.98 (*s*, 1H, CHO).

4-*Methoxy*-3,5-*diacetoxybenzaldehyde*. IR ν_{max} cm^{-1}: 2730 and 1695 (CHO); positive test with 2,4-DNPH; ^{1}H NMR ($CDCl_3$) (60 MHz): δ2.34 (*s*, 6H, 2 × AcO), 3.88 (*s*, 3H, OMe), 7.50 (*s*, 2H, H-2 and H-6) 9.84 (*s*, 1H, CHO).

3,5,3′,5′-*Tetraacetoxy*-4-*methoxybibenzyl*. *Trans*-3,5,3′,5′-tetraacetoxy-4-methoxystilbene (99 mg, 0.22 mmol) in EtOH (10 ml) was hydrogenated over PtO_2 (22 mg) for 8 hr to give crystalline 3,5,3′,5′-tetraacetoxy-4-methoxybibenzyl (88 mg), mp 92–94° (from EtOH); (Found: C, 62.17; H, 5.14. Calc. for $C_{23}H_{24}O_9$: C, 62.16; H, 5.40%); IR ν_{max} cm^{-1}: 2960, 2940, 2860, 1780 and 1760 (CO of phenolic acetate), 1620, 1590, 1500, 1450, (aromatic), 1370 (Me), 1205, 1190, 900, 885 (substituted ring); ^{1}H NMR ($CDCl_3$) (60 MHz): δ2.37 and 2.40 (2*s*, 12H, 4 × AcO), 2.9 (*s*, 4H, $2CH_2Ar$), 3.9 (*s*, 3H, OMe), 7.05 (*s*, 5H, aromatic); MS *m/z* (rel. int.): 444 [M]$^+$ (1), 402 [M − 42]$^+$ (17), 360 [M − 2 × 42]$^+$ (51), 318 [M − 3 × 42]$^+$ (20), 276 [M − 4 × 42]$^+$ (10), 153 (79), 122 (9), 43 (100).

Trans-3,4,5,3′,5′-*pentamethoxystilbene* (**3c**) [4]. Trans-3,5,3′,5′-tetrahydroxy-4-methoxystilbene (50 mg, 0.18 mmol) in MeOH was treated with CH_2N_2–Et_2O to give pale yellow rods of 3,4,5,3′,5′-pentamethoxystilbene (58 mg), mp 134–135° (from Et_2O–MeOH); IR ν_{max} cm^{-1}: 3010, 2990, 2940, 2840, 1590, 1500, 1465, 1435, 1430, 1150, 1125, 965 (*trans*-CH=CH), 835, 680 (substituted ring); ^{1}H NMR ($CDCl_3$) (60 MHz): δ3.85, 3.90, 3.95 (3*s*, 15H, 5 × OMe), 6.65 (*t*, 1H, J = 2 Hz, H-4′), 6.95 (*d*, 2H, J = 2 Hz, H-2′ and H-6′), 7.05 (*s*, 2H, CH=CH), 7.28 (*s*, 2H, H-2 and H-6).

Isolation of acidic components. From the EtOH extract an acidic fraction (8.6 g) ($NaHCO_3$) was isolated and analysed as acetate methyl esters. Acidic components (500 mg) were refluxed with MeOH and H_2SO_4 to give methyl esters (510 mg). These were treated with Ac_2O and pyridine to yield methyl ester acetates (526 mg) which were chromatographed on a column of silica gel (16 g). Hexane–Et_2O (1:3) eluted successively methyl *p*-acetoxybenzoate (180 mg), mp 85°; methyl 3,5-diacetoxy-4-methoxybenzoate (70 mg), mp 68–69°; and methyl 4-acetoxy-3,5-dimethoxybenzoate (80 mg), mp 129°. These derivatives of *p*-hydroxybenzoic acid and 3,5-dihydroxy-4-methoxybenzoic acid were characterized by IR and NMR.

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