Phytochemistry, Vol. 21, No. 7, pp. 1755–1758, 1982.
Printed in Great Britain.

0031-9422/82/071755-04$03.00/0

DIMERIC PHENOLIC CONSTITUENTS FROM THE ROOTS OF *TAMARIX NILOTICA*

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(*Revised received* 9 *November* 1981)

Key Word Index—*Tamarix nilitoca*; Tamaricaceae; roots; lignan; (±)-syringaresinol; ellagic acid 3,3'-dimethyl ether 4-*O*-β-D-glucopyranoside; NMR.

Abstract—The debarked roots of *Tamarix nilotica* contain the furanofuran lignan (±)-syringaresinol so far not reported from the Tamaricaceae, and the new natural product ellagic acid 3,3'-dimethyl ether 4-*O*-β-D-glucopyranoside. Further constituents were isoferulic acid, gallic acid, dehydrodigallic acid and ellagic acid. The structure of the isolated compounds was determined mostly by ^{1}H and ^{13}C NMR spectroscopy.

INTRODUCTION

Tamarix plants are distributed from Morocco to India. Extracts have been used in traditional Egyptian medicine, especially as antiseptic agents. They are also used for tanning and dyeing purposes. The polyphenolics and flavonoids of *T. aphylla* and *T. nilotica*, which grow wild in Egypt have been investigated [1, 2]. The study of the leaf chemical constitution of *T. nilotica*, led to the isolation and identification of the 3 - *O* - glucoside of the rare flavonol kaempferol - 7,4'-dimethyl ether [1].

Here we report on the occurrence of some phenolic constituents in the roots of *T. nilotica* which have not previously been investigated with respect to their chemical constitution. From the benzene extract the furanofuran lignan (±) - 2_e,6_e - bis - (3,5 - dimethoxy-4 - hydroxyphenyl) - 3,7 - dioxabicyclo - (3,3,0 - octane (syringa-resinol) (**1**) and isoferulic acid (**2**) was isolated. From the ethyl acetate extract of the plant we isolated the new natural product ellagic acid 3,3'-dimethyl ether 4 - *O* - β - D - glucopyranoside (**5**), gallic acid (**3**), dehydrodigallic acid (**4**) and ellagic acid (**6**). This is the first report of the occurrence of a lignan in the Tamaricaceae. The isolated new natural product (**5**) is of special interest because it is glycosylated through a phenolic hydroxyl group as in flavonoid glycosides [3] and not through a carboxyl group as is the case of ellagitannins [4].

RESULTS AND DISCUSSION

The meal of the debarked roots of *T. nilotica* was successively extracted with petrol, benzene and ethyl acetate. The petrol extract was shown on TLC to be a complex mixture of several compounds and was not further investigated. The benzene extract was fractionated into non-acidic and acidic fractions. Repeated prep. TLC led to the isolation of (±)-syringaresinol (**1**) from the former fraction and isoferulic acid (**2**) from the latter. Polyamide CC of the ethyl acetate extract, using water–methanol mixtures of decreasing polarity for elution, afforded the new natural product (**5**) in addition to gallic acid (**3**) dehydrodigallic acid (**4**) and ellagic acid (**6**).

(±)-Syringaresinol (**1**) was isolated as fine crystals which exhibited no optical activity when dissolved in chloroform. Its chromatographic properties and UV spectrum (Table 1) are similar to those of furanofuran lignans [5]. The mass spectrum shows a signal at *m/z* 418 $[M]^+$ and two signals at *m/z* 181 and 167, indicating the presence of a syringyl moiety [6]. In the ^{13}C NMR spectrum the chemical shifts of the furanofuran moiety (Table 2) are very close to those reported for the same moiety in other equatorial substituted 2,6-diaryl furanofuran lignans [7]. The introduction of a methoxyl group in vanillic acid [8] to form syringic acid results in ^{13}C NMR chemical shift changes which were applied to pinoresinol [9] to assign the signals of the syringyl moiety. Finally, the ^{1}H NMR spectrum of **1** was identical to that reported for syringaresinol [10].

The identification of **2**–**4** and **6** was achieved as described by one of us in a previous study [11]. The ^{13}C NMR spectra of **3** and **4** were recorded and assigned for the first time (Table 2). The ^{13}C NMR signals of **3** were assigned by applying the substituent rules on the ^{13}C NMR data of protocatechuic acid [8]. This assignment was used as the basis for analysing the ^{13}C NMR spectrum of **4** and of the new compound **5**. Unambiguous assignments could be achieved only by measuring the ^{13}C–^{1}H coupling constants. The ^{13}C NMR spectrum of **4** showed all of the 14 expected signals from which the low field signals were assigned to the non-equivalent carboxyl carbons because of their different splittings (*t*, *J* =

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4.4 Hz and *d*, $J = 4.94$ Hz, respectively). The signals for the tertiary carbons C-2, C-6 and C-6′ were identified by their one-bond coupling and the additional three-bond coupling for C-2 and C-6. Three-bond couplings were also found for C-4 (*t*), C-2′ and C-4′ (*d*). C-1′ and C-5′ showed only one-bond coupling ($J = 1.5$ and 3.5 Hz, respectively), whereas C-1 was split by two protons (*t*, $J = 1.6$ Hz). From the remaining three carbons, C-3 and C-5 exhibit a *dd* owing to a two-bond ($J = 2.9$ and 3.4 Hz) and four-bond coupling ($J = 1$ Hz), and C-3′ is coupled to only one proton over four bonds ($J = 1$ Hz). In addition, the ^{1}H NMR spectrum of **4** showed signals for three aromatic protons (one *s* and two *d*). The chemical shift difference of δ 0.52 between the two doublets was larger than that expected from the substitutent rules. Therefore, the upfield shift of H-2 may be attributed to the anisotropy of the second aromatic ring being out of the plane with the first ring.

Ellagic acid 3,3′ - dimethyl ether 4 - *O* - *β* - D - glucopyranoside (**5**) was separated as fine colourless crystals. On PC (R_f values, Table 1) it exhibited a

Table 1. UV and chromatographic properties of the investigated dimeric compounds of *Tamarix*

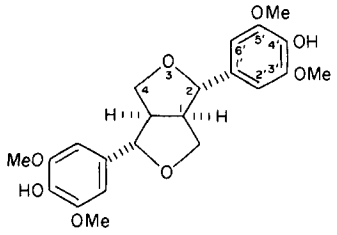
Compound	Chromatographic R_f (×100) H_2O	HOAc	HOAc	BAW	UV λ_{max}^{MeOH} nm
(±)-Syringaresinol (**1**)	72	77	91	90	227, 238*, 280, 308*
Isoferulic acid (**3**)	37	45	88	92	240, 295, 325
Gallic acid (**3**)	53	59	75	78	272
Dehydrodigallic acid (**4**)	54	60	72	75	272
Ellagic acid 3,3′-dimethyl ether-4-*O*-*β*-D-glucopyranoside (**5**)	23	36	61	58	260, 291*, 356, 374*
Ellagic acid 3,3′-dimethyl ether (**5a**)	0	9	82	91	251, 362*, 375
Ellagic acid	0	9	46	48	255, 362

*Shoulder.

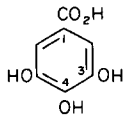
Table 2. ^{13}C chemical shifts, multiplicities and coupling constants (Hz) of syringaresinol (**1**), gallic acid (**3**), dehydrodigallic acid (**4**), ellagic acid 3,3′-dimethyl ether (**5a**) and ellagic acid 3,3′-dimethyl ether 4-*O*-*β*-D-glucopyranoside (**5**)*

Carbon No.	1	3	4	5a	5
1	54.3	120.6	120.6 (*t* 1.6)	111.8 (*d* 6.1)	114.7 (*d* 7.0)
2	86.1	108.8	111.3 (*d* 165, *d* 7.5)	141.1 (*s*)	141.9 (*s*)
3	71.8	145.5	148.0 (*d* 1.0, *d* 3.4)	140.2 (*m*)	142.5 (*m*)
4	71.8	138.1	139.6 (*t* 7.0)	153.0 (*s*)	151.9 (*s*)
5	—	—	146.1 (*d* 1.0, *d* 2.9)	111.4 (*d* 166)	112.7 (*d* 167.2)
6	—	—	107.1 (*d* 165.5, *d* 7.0)	112.0 (*s*)	113.0 (*s*)
7	—	167.7	168.2 (*t* 4.4)	158.3 (*s*)	159.0 (*s*)
8	—	—	—	—	—
1′	132.1	—	115.7 (*d* 1.5)	—	111.6 (*d* 7.0)
2′	102.7	—	136.6 (*d* 12.5)	—	141.3 (*s*)
3′	147.1	—	140.0 (*d* 1.0)	—	140.8 (*m*)
4′	134.3	—	139.7 (*d* 10.4)	—	152.1 (*s*)
5′	—	—	143.0 (*d* 3.5)	—	112.2 (*d* 168.5)
6′	—	—	109.0 (*d* 166)	—	112.2 (*s*)
7′	—	—	167.1 (*d* 4.9)	—	159.0 (*s*)
1″	—	—	—	—	101.9 (*d* 162.4)
2″	—	—	—	—	73.7 (*d* 145.3, *t* 3.7)
3″	—	—	—	—	76.8† (*d* 140.4)
4″	—	—	—	—	70.0 (*d* 145.3)
5″	—	—	—	—	77.5† (*d* 140.4)
6″	—	—	—	—	61.3 (*t* 141.0, *d* 4.9)
MeO	—	—	—	60.9 (*q* 146)	MeO-3-62.3 (*q* 147.6) MeO-3′- 61.7 (*q* 146.5)

*Numbering of carbons according to the given formulae.
†Assignment may be reversed.



1, (+)-enantiomer



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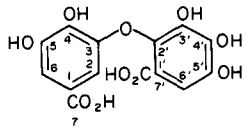

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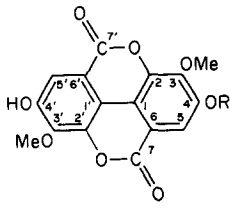

5, R = β-D-glucopyranoside
5a, R = H

bluish-white UV fluorescence which changed to bright yellow with ammonia. On normal acid hydrolysis it yielded glucose (co-PC) and the aglucone **5a**. The latter was also liberated from **5** on β-glucosidase hydrolysis and gave data [mp, chromatographic properties and UV maxima (Table 1), mass and IR spectra] identical to those reported for ellagic acid 3,3' - dimethyl ether [12]. The hypsochromic shift of the UV spectral maxima of **5** (Table 1) compared with those of **5a**, together with the other data showed that the isolated compound must be an ellagic acid 3,3' - dimethyl ether - *O* - β - D - glucoside. Establishment of the final structure of **5** was then achieved through ^1H (see Experimental) and ^{13}C NMR spectroscopy. The coupled ^{13}C NMR spectrum of **5a** showed one doublet for C-5 ($J = 166$ Hz), one doublet for C-1 ($J = 6.1$ Hz) and a complex pattern for C-3 due to coupling with the methoxyl group protons and with H-5. The remaining carbon signals were assigned by applying the substituent rules to the data of gallic acid (δ C-4 ≫ δC-2 ≫ δ C-6). Comparison of this spectrum with that of **5** reveals that the number of signals in the aromatic region has been doubled in the latter spectrum. The additional signals in the region of aliphatic carbons in the spectrum of **5** could be identified as a β-glycopyranose substituent. From hydrolysis, which yielded **5a**, and the number of carbon signals in the spectrum of **5** it is concluded that there is only one glucose moiety bound to one of the free hydroxyl groups. The assignment of the carbon signals of **5** (Table 2) was carried out in the same way as for **5a**, taking into consideration that changes in one ring would affect the chemical shifts of the carbons of this ring more than the chemical shifts of the carbons of the other. The chemical shift changes of the aromatic carbons due to introduction of the glucose moiety are in agreement with those reported for quercetin - 4' - *O* - glucoside [13].

EXPERIMENTAL

^1H chemical shifts were measured relative to TMS and ^{13}C chemical shifts relative to DMSO-d_6 and converted into the TMS scale by adding δ 39.5. Typical conditions: spectral width 5000 Hz, 8 K data points and a flip angle of 45°. ^1H-^{13}C coupled NMR spectra were obtained by the gated decoupling technique. TLC was carried out on Si gel 60 plates of 20 mm thickness for normal investigation and of 25 mm thickness for prep. TLC. Solvent systems for TLC: (1) EtOAc-*n*-C_6H_{14} (1:1); (2) MeOH-$CHCl_3$ (1:9). PC was carried out on a Whatman No. 1 paper. Solvent systems for PC: (3) H_2O; (4) HOAc (HOAc-H_2O, 3:17); (5) HOAc (HOAc-H_2O, 3:2); (6) BAW (*n*-BuOH-HOAc-H_2O, 4:1:5, upper layer).

The dried ground root material was extracted successively with petrol (45–60°), C_6H_6 and EtOAc. The solvents were removed under red. pres. and the dried extracts separately processed. The C_6H_6 extract was resolved into non-acidic and acidic fractions by dissolving in aq. $NaHCO_3$ soln (5%) at room temp. and extracting the non-acidic fraction with $CHCl_3$. The acidic fraction was obtained by extraction of the acidified (0.1 N HCl) aq. soln with $CHCl_3$. Prep. TLC afforded the following compounds.

(±)-*Syringaresinol* (**1**). Non-acidic fraction; mp (uncorr.) 174° (lit. [14] 174°); optically inactive. R_f values: (1) 30 and (2) 72; UV spectral data: Table 1; MS, *m/z*(%): 418 $[M]^+$ (100), 403 (6), 387 (11), 319 (7), 264 (6), 251 (13), 236 (18), 210 (20), 193 (26), 182 (52), 181 (89), 167 (67), 161 (30), 154 (25); ^1H NMR: δ 6.52 (4H, *s*, H-2', H-6' H-2'' H-6''), 5.44 (2H, *br s*, two phenolic groups), 4.68 (2H, *d*, $J = 5$ Hz, H-2_a, H-6_a), 4.25 (2H, *dd*, $J = 9$ and 6 Hz, H-4_e, H-8_e), 3.84 (12 H, *s*, OMe-4), 3.7–4.0 (2H, *m*, H-4_a, H-8_a), 3.08 (2H, *m*, H-1, H-5); ^{13}C NMR see Table 2.

Isoferulic acid (**2**). Acidic fraction; mp (uncorr.) 231°; R_f values and UV spectral data: Table 1; acetylation of **2** (Ac_2O-pyridine) yielded a colourless crystalline acetyl derivative, mp 198° (uncorr.) which was not affected on admixture with authentic sample [11].

The EtOAc extract was applied to a polyamide column and eluted by H_2O followed by H_2O-MeOH mixtures of decreasing polarities to yield three major fractions. **3–6** were then isolated as follows.

Gallic acid (**3**). Isolated from the 20% aq. MeOH column fraction; mp and mmp 250°; R_f values and UV spectral data: Table 1. ^1H NMR: δ 6.98 (2H, *s*, H-2, H-6); ^{13}C NMR: Table 2.

Dehydrodigallic acid (**4**). Isolated from the 40% aq. MeOH column fraction; mp and mmp 360°; R_f values and UV spectral data: Table 1. **4** yielded gallic acid on drastic alkaline hydrolysis (2N aq. NaOH, 100°, 2 hr) and yielded 4,5,3',4',5' - pentahydroxyxanthone 1-carboxylic acid (mp uncorr. 220°) on dehydration by conc. H_2SO_4 [11]. ^1H NMR: δ 7.02 (*d*, $J = 2.5$ Hz, H-2), 6.5 (*d*, $J = 2.5$ Hz, H-6), 6.9 (*s*, H-6'); ^{13}C NMR: Table 2.

Ellagic acid 3,3' - *dimethyl ether* - O - β - D - *glucopyranoside* (**5**). Separated from the 60% aq. MeOH column fraction; mp (uncorr.) 297° (decomp.); R_f values and UV spectral data: Table 1; ^1H NMR: δ 7.78 (1H, *s*, H-5), 7.6 (1H, *s*, H-5'), 5.12 (not well resolved *d*, half-width 10 Hz, H-1'' 4.16 (3H, *s*, OMe-3), 4.1 (3H, *s*, OMe-3'), 3.28–3.6 (6H, *m*, glucopyranosyl moiety); ^{13}C NMR: Table 2. **5** yielded glucose and ellagic acid 3,3'-dimethyl ether (**5a**) on acid hydrolysis (1.5 N aq. HCl, 100°, 45 min). The latter compound was also released on β-glucosidase hydrolysis of **5**.

Ellagic acid 3,3'-*dimethyl ether* (**5a**). Mp (uncorr.) 333° (lit. [12], 333–335°); R_f values and UV spectral data: Table 1; IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2960, 2840 (OMe), 1730 (C=O); MS, *m/z*(%): 330 $[M]^+$ (100), 315 (38), 287 (10), 259 (3), 231 (3.5), 203 (5), 103 (6); ^1H NMR: δ 10.6 (*br s*, OH-4, OH-4'), 7.52

(2H, *s*, H-5, H-5'), 4.08 (6H, *s*, OMe-3, OMe-3'); ^{13}C NMR: Table 2.

Ellagic acid (**6**). Isolated from the 90% aq. MeOH column fraction; mp uncorr.) 360°; R_f values and UV spectral data: Table 1. Methylation of **6** with CH_2N_2–Et_2O[11] yielded 3,4,3',4'-tetramethoxyellagic acid (mp uncorr. 344°, decomp.).

Acknowledgements—This work was supported by a grant awarded by the Humboldt Foundation, Bonn, Germany, to M. A. M. N. We thank H. Herzog, Dortmund, for technical assistance.

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