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3,4,8,9,10-PENTAHYDROXY-DIBENZO[*b*, *d*]PYRAN-6-ONE FROM *TAMARIX NILOTICA*

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Key Word Index—*Tamarix nilotica*; Tamaricaceae; flowers; phenolic lactones; 3,4,8,9,10-pentahydroxy-dibenzo[*b*, *d*]pyran-6-one; ellagic acid; structural determination.

Abstract—A new natural product, 3,4,8,9,10-pentahydroxy-dibenzo-[*b*, *d*]pyran-6-one was isolated from the flowers of *Tamarix nilotica* which also contains the known compound ellagic acid, 2,3,7,8-tetrahydroxy [1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,10-dione. The structure of the new compound was determined by chemical and spectroscopic methods. The ^{13}C MNR spectrum of ellagic acid was recorded and assigned.

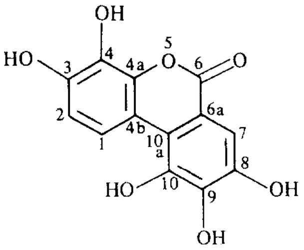
INTRODUCTION

The aqueous acetone extract of the flowers of *Tamarix nilotica* was reported by us to contain the methyl and ethyl esters of gallic acid, *p*-methoxygallic acid, kaempferol and quercetin 3-*O*-glucuronides, the 3-*O*-sulphated kaempferol 7,4′-dimethyl ether and the free flavonols, kaempferol, quercetin and kaempferol 7,4′-dimethyl ether beside the digalloylglucose, niloticin [1, 2]. Further investigation of this extract afforded the new natural phenolic lactone, 3,4,8,9,10-pentahydroxy-dibenzo[*b*, *d*]pyran-6-one (1) and ellagic acid (**2**). In this paper we report the isolation and structural elucidation of **1**. Also, the ^{13}C NMR data of ellagic acid is reported for the first time. Compound **1** is another natural product added to the well known biosynthetic transformation products of hexahydroxydiphenic acid such as breviofolin and chebulic acid [3]. Also, it belongs to the 3,4-benzocoumarins, which are of rare natural occurrence.

RESULTS AND DISCUSSION

The last fraction, eluted with methanol from the polyamide column of the aqueous acetone extract of the flowers of *T. nilotica* [2], was shown by 2D-PC, in UV light to contain two fluorescent compounds **1** and **2**, apart from the yellow flavonols which were previously investigated [2]. Pure samples of **1** and **2** were isolated by applying a polyamide column fractionation of this methanolic fraction using ethyl acetate saturated with water as an eluent.

Compound **1** was isolated as colourless needles which gave a molecular formula $C_{13}H_8O_7$ by high resolution mass spectrometry. On PC, **1** appeared as a fluorescent blue spot in UV light. It gave a blue ferric chloride reaction and remained unchanged after acid or alkaline hydrolysis. On alkali-fusion it yielded 4,5,6,2′,3′,4′-hexahydroxybiphenyl (co-PC and UV data). The IR spectrum of **1** showed strong absorption at 3400, 1695, 1630 and



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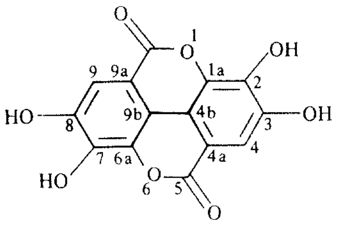

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1610 cm^{-1}, consistent with the presence of phenolic hydroxyl, α-pyrone C=O and benzenoid C=C groups, respectively. The mass spectrum showed an $[M]^+$ at *m/z* 276 and a sequential loss of 28 mu fragments, a fragmentation pattern which is in agreement with that reported for coumarins bearing oxygen functions [4]. The chemical and spectral (UV, IR and mass spectrum) analytical data of **1** were found to be closely similar to those given for the benz α-pyrone coumarins [4] as well as to those reported for ellagic acid derivatives [5], and suggested a pentahydroxy 3,4-benzocoumarin structure for **1**. The identity of **1** as 3,4,8,9,10-pentahydroxy-dibenzo[*b*, *d*]pyran-6-one was achieved by comparison with an authentic sample prepared from ellagic acid as described by Perkin *et al.* [6] (mp, mmp, R_f-values and UV data, see Experimental). A study of the ^1H NMR of **1** lent further support to this view. The spectrum showed two doublets at $\delta 6.8$ and 8.44 assignable to the two *ortho* aromatic protons at C-1 and C-2 in addition to one singlet at $\delta 7.46$ assignable to the aromatic proton at C-7 (see formula). In the ^{13}C NMR spectrum of **1**, the assignment of the recorded signals as aided by comparison with the ^{13}C NMR data of ellagic acid (see Experimental) and hexahydroxydiphenic acid dimethyl ester [7]. The spectrum showed 11 signals assigned as follows: one signal at $\delta 161.58$, assignable to the carbonyl carbon C-6, six signals in the region from $\delta 133.07$–151.47 assignable to the oxygenated carbons and four signals in the region from $\delta 108.8$ to 118.4 assignable to the protonated and quaternary carbons. The ^{13}C NMR data thus confirmed the structure of **1** to be 3,4,8,9,10-pentahydroxydibenzo[*b*, *d*]pyran-6-one.

Compound **2** was obtained as faint yellow crystals identical with ellagic acid (mp, mmp, R_f-values and UV data) [8]. It had an MW of 302 as shown by FAB-mass spectrometry and its IR spectrum showed absorption at 3380, 1720, 1690 and 1610 cm^{-1}, thus suggesting the presence of phenolic hydroxyl, α-pyrone C=O and benzenoid C=C groups. The symmetrical molecule of **2** gave a ^1H NMR spectrum which contained only one singlet at $\delta 7.5$ assignable to the two identical protons at C-4 and C-9 (see formula). In the ^{13}C NMR spectrum of **2** assignments of the recorded signals (see Experimental) was achieved by applying the substituent rules on the ^{13}C NMR data of 2,7-dimethoxy ellagic acid (3,8-dihydroxy-2,7-dimethoxy-[1]benzopyrano[5,4,3-cde] [1]benzopyran-5,10-dione) [8].

EXPERIMENTAL

NMR spectra were measured at 100 MHz and chemical shifts were measured relative to TMS. ^{13}C NMR chemical shifts were relative to DMSO-d_6 and converted into the TMS scale by adding 39.5. Typical conditions: spectral width 5000 Hz 8K data points and a flip angle of 45°. PC was carried out on Whatman No. 1 using solvent systems 1–3: 1, H_2O; 2, HOAc–H_2O (3:17); 3, BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper layer).

Plant material and fractionation. An aq. Me_2CO extract of the flowers of *T. nilotica* was worked up as reported in ref. [2].

Isolation and identification. The last fraction, eluted with MeOH from the polyamide column of the aq. Me_2CO extract was dried under vacuum and applied to a sub-column using polyamide (polyamide 6 S for CC) as adsorbent and EtOAc satd with H_2O for elution. Compounds **1** and **2** were individually eluted in the last two successive fractions.

3,4,8,9,10-*Pentahydroxy-dibenzo*[*b*, *d*]*pyran*-6-*one* (**1**). Purified by crystallization (twice) from Me_2CO, mp 318°; R_f-values: 00 (H_2O), 22 (HOAc), 46 (BAW); UV λ_{max}^{MeOH} nm: 219, 264, 287 (inf), 349 (inf). Acid hydrolysis (2 N aq. HCl, 100°, 7 hr) or alkaline hydrolysis (5% aq. KOH, 100°, 3 hr) had no effect on **1**. It yielded 4,5,6,2′,3′,4′-hexahydroxybiphenyl when fused with KOH at 245° for 3 min. Fusion product: R_f-values: 44 (H_2O), 56 (HOAc), 41 (BAW); UV λ_{max}^{MeOH} nm: 259. IR of **1**: ν_{max} cm^{-1}: 3400, 3300, 2910, 2850, 1690, 1610, 1590; MS *m/z* (rel. int.): 276 (100, $[M]^+$), 248 (8), 247 (10), 219 (15), 191 (10), 163 (12), 115 (10), 89 (12), 73 (15), 55 (18); ^1H NMR: $\delta 8.44$ (*d*, $J = 9$ Hz, H–1), 6.8 (*d*, $J = 9$ Hz, H-2), 7.46 (*s*, H-7); ^{13}C NMR: $\delta 161.58$ (C=O), 151.47, 146.4, 145.98, 143.55, 140.62, 133.07 (C-3, C-4, C-4_a, C-8, C-9, C-10), 118.4, 112.46, 112.06, 108.8 (C-6_a, C-7, C-10_a, C-4_b).

Ellagic acid (**2**). Purified by crystallization from pyridine, mp > 360°; R_f-values: 00 (H_2O), 09 (HOAc), 48 (BAW); UV λ_{max}^{MeOH} nm: 255, 362. On alkali-fusion (KOH, 245°, 3 min) **2** yielded 4,5,6,2′,3′,4′-hexahydroxybiphenyl, identified as given above. It yielded **1** on boiling with 75% aq. KOH for 10 min. IR of **2**: ν_{max} cm^{-1}: 3380, 3270, 1720, 1690, 1610, 1575, 1485; FAB-MS (mu): 302 ($[MH]^+$, 303); ^1H NMR: $\delta 7.5$ (*s*, H-4 and H-9); ^{13}C NMR: $\delta 158.8$ (C=O), 148 (C-3 and C-8), 139.2 (C-2 and C-7), 136.6 (C-1_a and C-6_a), 110.6 (C-4, C-4_a, C-9 and C-9_a), 107.3 (C-4_b and C-9_b).

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