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ALKALOIDAL, LIGNAN AND PHENOLIC CONSTITUENTS OF *EPHEDRA ALATA*

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Key Word Index—*Ephedra alata*; Ephedraceae; alkaloid; ephedralone; 7-methoxy-4-quinolone 2-carboxylic acid; lignan; (±)-syringaresinol; phenolic; nilocitin; 2,3-digalloylglucopyranose.

Abstract—In addition to the known *p*-coumaric acid, the furanofuran lignan (±)-syringaresinol and the digalloylglucose, nilocitin, were obtained from the whole plant of *Ephedra alata*. A new natural alkaloid, ephedralone, was also isolated. The structures were determined mostly by mass, ^{1}H and ^{13}C NMR spectroscopy.

INTRODUCTION

During the course of our investigation on *Ephedra alata*, we have reported seven flavonoids, which were either *C*-glycosylflavones or flavonol *O*-glycosides [1]. Here we report the isolation of *p*-coumaric acid (**1**) and (±)-2,6-bis-(3,5-dimethoxy-4-hydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (syringaresinol, **2**) from the chloroform extract, the 2,3-digalloylglucopyranose (nilocitin, **3**) from the ethanolic extract and the new alkaloid 7-methoxy-4-quinolone 2-carboxylic acid (ephedralone, **4**) from the aqueous extract of the same plant. This is the first reported occurrence of a lignan in the Ephedraceae. Also, it is the second report about the natural occurrence of nilocitin, which was previously, isolated by us [2] from *Tamarix nilotica*. The isolated new alkaloid is of special interest because it represents the first quinoline alkaloid to be present in Ephedraceae, which is well known as a source of ephedrine alkaloids [3]. 4-Quinolones are of rare natural occurrence and the majority of them have been found in the family Rutaceae [4] or in bacteria [5].

RESULTS AND DISCUSSION

Proceeding as described in ref. [1], petrol, chloroform, ethyl alcohol and water extracts were obtained. Silica gel CC of the chloroform extract, using chloroform as an eluent, followed by partition of the desorbed phenolic fraction into acidic and non-acidic portions led to the isolation of **1** from the former and **2** from the latter. Polyamide CC of each of the ethyl alcohol extract and the water extract, using methanol–water mixtures of decreasing polarities, led to the isolation of **3** from the ethyl alcohol extract and **4** from the water extract.

Compound **1** was identified by mp, CoPC, UV and ^{1}H NMR data as *p*-coumaric acid. Compound **2** was identified by measurements of optical activity, CoPC, UV, mass and ^{1}H NMR spectral analysis as syringaresinol. The identity was confirmed through ^{13}C NMR spectral analysis. Compound **3** exhibited an M_r of 484 mU (pos. FAB–MS). It was identified by CoPC, UV spectral data and hydrolytic procedures as 2,3-digalloylglucose (nilocitin). The identity was confirmed by ^{1}H and ^{13}C NMR spectral analysis.

Compound **4** was isolated as colourless prisms from water–methanol. Accurate mass measurements established the molecular formula as $C_{11}H_9NO_4$ (*m/z* 219, M^+). Compound **4** appeared on PC as a fluorescent blue spot lacking phenolic character (negative $FeCl_3$ reaction). Its UV spectrum in methanol showed two main absorption maxima at 252 and 332 nm. The latter showed a bathochromic shift of 28 nm with NaOMe, and a hypsochromic shift of 17 nm on addition of 1 N HCl. These are closely similar to 4-quinolones [6] substituted at position 2 with substituents which react with alkali. The IR spectrum of **4** disclosed two carbonyl bands at 1730 and 1640 cm^{-1}, consistent with a carboxyl carbonyl and a quinolone carbonyl groups, respectively. The spectrum also showed a band at 800 cm^{-1}, consistent with a 1,2,4-trisubstituted benzene. Other bands in this spectrum are similar to those reported for 4-quinolones [7]. Compound **4** resisted acid and alkaline hydrolysis and yielded on silylation a disilyl derivative of M_r 363 (*m/z*). ^{1}H NMR spectroscopy proved the presence of 1,2,4-trisubstituted benzene, whereby three signals, each integrated to one proton, were observed at δ7.28 (*d*, *d*, J = 9 Hz and J = 2.5 Hz), 7.46 (*d*, J = 2.5 Hz) and 7.86 (*d*, J = 9 Hz). The spectrum also shows a methoxyl signal at δ3.87 and a singlet of one olefinic proton at δ6.6. These data together proved **4** to be 7-methoxy-4-quinolone 2-carboxylic acid.

The EI-mass spectrum of **4** lent support to this view and showed a fragmentation which was found consistent with those reported for 4-quinolones bearing 2-carboxylic acid residues [8]. The fragmentation proceeded through loss of H_2O from the molecular ion (*m/z* 219, M^+) followed by the successive loss of two CO to give a 145 *m/z* peak, corresponding to a methoxy indole fragment. The spectrum also showed loss, from the molecular ion of CO_2 group to give 175 *m/z* peak and of CH_2O group to give 189 *m/z* peak which proved the presence of a carboxylic and methoxyl functions in the molecule. For further confirmation the ^{13}C NMR spectrum of **4** was recorded

and assigned (see Experimental). The recorded spectrum showed ten signals, among which two carbonyl signals at 164.05 and 177.4 ppm were assigned to the carboxylic carbon and the quinolone carbonyl carbon, respectively. The methoxyl carbon signal was located at 55.3 ppm. Other signals in this spectrum were assigned by comparison with the ^{13}C NMR data of appropriate model compounds such as *o*-aminoacetophenone and the 4-quinolone alkaloid, lemobiline [9, 10].

EXPERIMENTAL

^1H NMR chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts 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°. FAB–MS were recorded on a MM7070 E instrument (VG Analytical). PC was carried out on Whatman paper No. 1 using solvent systems: 1—H_2O; 2—HOAc (HOAc–H_2O, 3:17); 3—BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer); 4—BPOH (C_6H_6–*n*-BuOH–C_6H_5N–H_2O, 1:5:3:3, top layer). Solvent systems 3 and 4 were used for sugar analysis.

Plant material and isolation. $CHCl_3$, EtOH and H_2O extracts of the whole *E. alata* were prepared as described in ref. [1].

Identification. The $CHCl_3$ extract was applied to a silica gel column and eluted with $CHCl_3$ to isolate the phenolic components which were desorbed in the last column fraction. The phenolic eluate was concentrated *in vacuo* and treated with 5% aq. $NaHCO_3$. The separated aq. layer was extracted after acidification, by ether to yield **1**. Crystallisation from $CHCl_3$–MeOH of the material left after removal of the solvent from the $CHCl_3$ layer afforded **2**. The EtOH extract was dried under vacuum, applied to a polyamide column and eluted with water followed by H_2O–MeOH mixtures of decreasing polarities. Pure **3** was isolated from the 4:1 fraction through a sub-polyamide column followed by precipitation of the desorbed impure **3**, dissolved in Me_2CO by Et_2O. The H_2O extract was subjected to polyamide column fractionation using H_2O–MeOH mixtures of decreasing polarities to yield **4** in the 2:3 fraction. Pure **4** was obtained by crystallisation from H_2O–MeOH.

p-Coumaric acid (**1**): R_f-values: 0.43 (H_2O), 0.45 (HOAc), 0.90 (BAW). UV λ_{max} nm MeOH: 226, 310; NaOMe: 228, 333.

(±)-Syringaresinol (**2**): mp (uncorr.) 174° (lit. [11], 174°); optical inactive in $CHCl_3$. R_f-values: 0.72 (H_2O), 0.77 (HOAc), 0.90 (BAW). UV λ_{max}^{MeOH} nm: 227, 238*, 280, 308*. EI–MS, *m/z* (%): 418 (100, M^+), 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). ^{13}C NMR: δ71.8 (C-1 and C-5), 86.1 (C-2 and C-6), 54.3 (C-4 and C-8); phenyl residue: δ131.2 (C-1′ and C-1″), 102.7 (C-2′, C-6′, C-2″ and C-6″), 147.1 (C-3′, C-5′, C-3″ and C-5″), 134.3 (C-4′ and C-4″).

Nilocitin (**3**): R_f-values: 0.60 (H_2O), 0.75 (HOAc), 0.50 (BAW). M_r 484 mU, pos. FAB–MS (MH^+: 485). UV λ_{max}^{MeOH} nm: 276. ^{13}C NMR: sugar moieties: α-glucose: δ89.3 (C-1), 72.2 (C-2), 72.2 (C-3), 68.3 (C-4), 72.2 (C-5), 60.6 (C-6); β-glucose: δ94.5 (C-1), 73.15 (C-2), 75.5 (C-3), 68.3 (C-4), 76.7 (C-5), 60.6 (C-6); galloyl moieties: δ165.5 and 165.4 (C=O carbons in α-isomer), 164.8 and 165.2 (C=O carbons in β-anomer); 120.6, 120.64, 121.38, 121.42 (C-1 of the galloyl moieties in both α- and β-anomers); 109.97 (C-2 and C-6 in all galloyl moieties); 145.6 (C-3 and C-5 in all galloyl moieties); 138.67 and 138.9 (C-4 in all galloyl moieties).

Ephedralone (**4**): mp (uncorr.) 268°. R_f-values: 0.47 (H_2O), 0.52 (HOAc), 0.78 (BAW). UV λ_{max}^{MeOH} nm: 212, 252, 260*, 300*, 332; +0.1 N HCl 260, 295*, 315; +NaOMe 217, 252, 258, 300*, 360. IR ν_{max} cm^{-1}: 3400, 2930, 2910, 2880, 1730, 1640, 1600, 1540, 1460, 1400, 1380, 1220, 1120, 1070, 1030, 800. EI–MS: *m/z* (%): 219 (30), 201 (4), 189 (6), 175 (100), 173 (18), 160 (14), 145 (24), 143 (7), 132 (32), 117 (8), 104 (26), 89 (7), 77 (12). EI–MS of disilyl derivative: *m/z* (%): 363 (22), 348 (100), 334 (23), 318 (20), 304 (9), 261 (52), 246 (8), 231 (8), 169 (24), 147 (9), 103 (9). ^1H NMR of **4**: δ3.86 (*s*, OMe), 6.6 (*s*, 3-H), 7.28 (*dd*, J = 7 Hz and J = 2.5 Hz, 6-H), 7.45 (*d*, J = 2.5 Hz, 8-H), 7.86 (*d*, J = 7 Hz, 5-H). ^{13}C NMR: δ177.4 (C-4), 164.05 (C-2′,C=O carboxylic), 136.7 (C-2), 103.5 (C-3 and C-8), 107.9 (C-6), 121.6 (C-10), 122.9 (C-5), 141.2 (C-9), 155.9 (C-7), 55.3 (OMe).

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