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FLAVONOIDS OF *IPHIONA SCABRA*

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Key Word Index—*Iphiona scabra*; Compositae; Inuleae; sulphated flavonoids; highly methoxylated aglycones.

Abstract—Thirteen flavonoids were isolated from the aerial parts of *Iphiona scabra*: the 3-sulphate, 3,4′-disulphate and 3,7,4′-trisulphate of isorhamnetin; the 3,7-disulphate and 3,7,4′-trisulphate of quercetin; and the 7-sulphate of hispidulin; the 3-glucosides and 3-galactosides of isorhamnetin and quercetin; and artemetin, salvigenin and 5-hydroxy-3,6,4′-tetramethoxyflavone.

INTRODUCTION

Iphiona Cass. (Compositae, tribe Inuleae, subtribe Inulinae) is a small genus of about ten species, which is distributed from North-East Africa to Central Asia [1]. Previously, *Iphiona* and *Pegolettia* were treated as one group; however, in a revision, Merxmuller [2] separated them. Recently, Leins [3] has supported Merxmuller's view on the basis of the pappus character and chromosome number of $2n = 18$ [4].

As a part of our biochemical systematic study of the subtribe Inulinae, we report here on *Iphiona scabra* DC., a species which afforded 13 flavonoids including three new sulphates.

RESULTS AND DISCUSSION

Column and preparative paper chromatography of the aqueous methanolic extracts of *I. scabra* led to the isolation of 13 flavonoids. The new compound **1** moved towards the anode in an electrophoretic system in agreement with trisulphate data [5, 6], and appeared deep purple on paper in UV light changing to brownish-green with ammonia, indicating 4′-*O*-substitution. The lack of change with the reagent Naturstoffreagenz A (NA) suggested that the compound lacks a free 3′,4′-dihydroxy system; this was confirmed by $AlCl_3$–HCl and NaOAc–H_3BO_3 spectra. The absence of both a bathochromic shift of band II in sodium acetate relative to band II in methanol and the presence of band III (at *ca* 320 nm) in sodium methoxide supported substitution at position 7. A bathochromic shift (45 nm) of band I in aluminium chloride relative to band I in MeOH–HCl suggested a free 5-hydroxyl and 3-substitution. The presence of 3-sulphate was also demonstrated by a bathochromic shift in the presence of hydrochloric acid. The ^{1}H NMR data (as the tetramethylsilyl ether in carbon tetrachloride) were similar to those for isorhamnetin: one-proton doublets at δ6.2 ($J = 2.5$ Hz) and 6.5 for H-6 and H-8, respectively; an *ortho*-coupled doublet at δ6.9 ($J = 9$ Hz) for H-5′, a double-doublet at about δ7.5 ($J = 9$ and 2.5 Hz) for H-6′, a narrow *meta*-coupled doublet at about δ7.4 for H-2′ and a three-proton singlet at δ3.9 for the 3′-methoxyl group. Acid hydrolysis of **1** yielded isorhamnetin while sulphate was detected by precipitation with barium chloride. A compound with the same spectral and electrophoretic properties as **1** was previously detected as a trace component in *Flaveria* [6].

Compound **2**, which was isolated from the 30% methanolic fraction, exhibited a lower electrophoretic mobility than **1**, suggesting a disulphate substitution. The colour reactions established a free 5-hydroxyl group and 3-substitution. The presence of 3-sulphate was also demonstrated by a bathochromic shift in the presence of hydrochloric acid. In the UV spectra of **2**, the bathochromic shift of band II in sodium acetate, relative to band II in methanol, supported a free 7-hydroxyl group. The change in colour on paper in UV light from deep purple to brownish-green with ammonia and a bathochromic shift with decrease in intensity of band I in the sodium methoxide spectrum demonstrated 4′-substitution. When **2** was hydrolysed with 0.1 N trifluoroacetic acid it yielded isorhamnetin and sulphate. Finally, ^{1}H NMR spectrum of **2** was similar to that of **1**; therefore **2** was identified as isorhamnetin 3,4′-disulphate, a new natural product.

The third new compound **3** contained an aglycone different from that of **1** and **2**. The structure of **3** was established as 6-methoxyapigenin 7-sulphate (hispidulin 7-sulphate) by the following spectral data. When the compound was viewed on paper in UV light it exhibited a purple colour change to greenish-yellow with ammonia and NA indicating a 5,4′-dihydroxy system. The absence of a bathochromic shift of band II in sodium acetate, relative to band II in MeOH–HCl, as well as the presence of band III in sodium methoxide supported a 7-substituent. A methoxyl function at C-6 was verified by the reduced bathochromic shift (20 nm) of band I in $AlCl_3$–HCl relative to band I in MeOH–HCl, and the presence of a singlet at δ6.5 in the ^{1}H NMR spectrum (as the tetramethylsilyl ether in carbon tetrachloride) for H-8 and a three-proton singlet at δ3.8 for the 6-methoxyl group. Furthermore, the mass spectral fragments $[M-15]^-$ and $[A-15]^-$, for the loss of a methyl, also supported this substitution. Additionally, ^{1}H NMR signals included a two-proton doublet for H-2′,6′ at δ7.4 ($J = 9$ Hz) coupled to another doublet at δ6.9 ($J = 9$ Hz) for H-3′,5′; H-3 appeared as a singlet at δ6.3. Finally, the

electrophoretic mobility was in agreement with a monosulphate.

Three additional sulphated compounds were isolated and from their spectral properties and by direct comparison with authentic samples they were identified as the 3,7,4′-trisulphate of quercetin (**4**), and the 3,7-disulphate (**5**) and 3-sulphate (**6**) of isorhamnetin. The three highly methoxylated aglycones and the four glycosides were identified as 5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone (artemetin) (**11**), 5-hydroxy-6,7,4′-trimethoxyflavone (salvigenin) (**12**), 5-hydroxy-3,6,7,4′-tetramethoxyflavone and the 3-*O*-*β*-D-glucoside and 3-*O*-*β*-D-galactoside of isorhamnetin and quercetin by UV, mass spectral and ^{1}H NMR spectral data. The glycosides were also identified by hydrolysis to the expected products.

In the positive FAB mass spectrum of isorhamnetin 3-sulphate (**6**), the $[M+H]^+$ ion observed at *m/z* 419 ($C_{16}H_{11}O_{10}SNa+H$) confirmed the presence of a sulphate moiety with a Na^+ counter-ion. Additionally, an $[M+Na]^+$ (i.e. two Na^+ counter-ions present) fragment at *m/z* 441 was found in the spectrum [7]. The negative FAB mass spectrum of the 3,4′-disulphate (**2**) and 3,7-disulphate (**5**) of isorhamnetin showed $[M-H]^-$ at *m/z* 535 ($C_{16}H_{10}O_{13}S_2Na+K+H$), indicative of two sulphate moieties with Na^+ and K^+ counter-ions. Moreover, $[M-K]^-$ and $[M-K-Na]^-$ were also observed at *m/z* 513 and 487, respectively. Although the negative FAB mass spectra of the 3,7,4′-trisulphate of isorhamnetin (**1**) and quercetin (**4**) are unclear, peaks at *m/z* 576 ($C_{16}H_9O_{16}S_3Na$) and 585 ($C_{15}H_7O_{16}S_3Na_2$), respectively, were observed, suggesting isorhamnetin trisulpate was complexed with only one Na^+ counter-ion while, on the other hand, quercetin trisulphate was associated with two Na^+ counter-ions. While different counter-ions have been reported from *Brickellia* [8], the occurrence of different counter-ions on the same molecule was totally unexpected, but this phenomenon has been observed in other studies (Cerny, R. L., personal communication). Furthermore, since most FAB mass spectral work has been recorded for monosulphates, published FAB mass spectral data for polysulphates, which are rare in nature, are currently unreported.

EXPERIMENTAL

Plant material. Aerial parts of *Iphiona scabra* were collected in Egypt, south of Sinai in March 1985. A voucher specimen (A. Ahmed No. 41), identified by Prof. Dr. El-Hadidi, Department of Botany, Cairo University, has been deposited at the Herbarium of the Department of Botany, El-Minia University.

General techniques. CC employed Polyclar AT, microcrystalline cellulose (Avicel) and Sephadex LH-20. Electrophoresis was carried on Whatman 3MM paper in a pH 1.9 buffer (HCO_2H–HOAc–H_2O, 33:147:1820). The solvent system for PC and TLC was TBA (*t*-BuOH–HOAc–H_2O, 3:1:1), *n*-BAW, upper layer, (*n*-BuOH–HOAc–H_2O, 4:1:5), 15% and 30% HOAc. Visualization of the flavonoids on PC and TLC was realized either by UV light + NH_3 or by spraying with NA in MeOH. All UV data were recorded using standard procedures [9]. ^{1}H NMR spectra of the TMSi ether of these flavonoids were recorded in CCl_4 at 90 MHz and are reported as *δ*-values (ppm) relative to TMS as internal standard. MS data were recorded by direct probe EIMS at 70 eV.

Isolation and characterization of flavonoids. The dried and ground leaves of *I. scabra* (300 g) were extracted 3 × each with 80% and 50% aq. MeOH. The extract, combined and concentrated, was chromatographed over Polyclar AT (GAF Corp.) eluted first with H_2O and then with increasing amounts of MeOH. The H_2O fraction yielded two components on Whatman 3MM chromatography paper developed 72 hr in TBA. The resulting bands yielded the 3,7,4′-trisulphates of isorhamnetin and quercetin. The 30% fraction afforded the 3,7-disulphate and 3,4′-disulphate of isorhamnetin. The 50% fraction gave isorhamnetin 3-sulphate and 6-methoxyapigenin 7-sulphate (hispidulin 7-sulphate), while the 70% fraction produced the four monoglycosides of isorhamnetin and quercetin. The methanolic fraction yielded the highly methoxylated aglycones.

All compounds were purified over Sephadex LH-20 prior to analysis by UV, ^{1}H NMR and MS [9]. Acid hydrolysis of glycosides (0.1 N TFA, 2 hr) yielded glucose, galactose, isorhamnetin and quercetin, all of which co-chromatographed with authentic samples.

Isorhamnetin 3,7,4′-trisulphate (**1**). R_f values: TBA, 0.05; 15% HOAc, 0.93. UV λ_{max}^{MeOH} nm: 282, 320 sh, 337; MeOH–HCl 257, 270, 310 sh, 360; NaOMe 282, 320 sh, 385; $AlCl_3$ 282, 302 sh, 345, 400; $AlCl_3$–HCl 277, 300 sh, 345, 400; NaOAc 275, 337; NaOAc–H_3BO_3 270, 312 sh, 342. ^{1}H NMR (as TMSi ether): *δ*6.2 (*d*, $J_{6,8}$ = 2.5 Hz, H-6), 6.5 (*d*, $J_{8,6}$ = 2.5 Hz, H-8), 6.9 (*d*, $J_{5',6'}$ = 9 Hz, H-5′), 7.4 (*d*, $J_{2',6'}$ = 2.5 Hz, H-2′), 7.6 (*dd*, H-6′), 3.9 (*s*, 3′-OMe).

Isorhamnetin 3,4′-disulphate (**2**). R_f values: TBA, 0.2; 15% HOAc, 0.75. UV λ_{max}^{MeOH} nm: 250, 270, 333; MeOH–HCl 255, 270 sh, 380; NaOMe 270 sh, 280, 360; $AlCl_3$ 260 sh, 280, 345, 395; $AlCl_3$–HCl 260 sh, 280, 345, 395; NaOAc 272, 360; NaOAc–H_3BO_3 250, 270, 335. ^{1}H NMR (as TMSi ether): *δ*6.2 (*d*, $J_{6,8}$ = 2.5 Hz, H-6), 6.5 (*d*, $J_{8,6}$ = 2.5 Hz, H-8), 6.9 (*d*, $J_{5',6'}$ = 9 Hz, H-5′), 7.4 (*d*, $J_{2',6'}$ = 2.5 Hz, H-2′), 7.6 (*dd*, H-6′), 3.9 (*s*, 3′-OMe).

Hispidulin 7-sulphate (**3**). R_f values: TBA, 0.65; 15% HOAc, 0.25. UV λ_{max}^{MeOH} nm: 280, 330; MeOH–HCl 280, 330; NaOMe 270, 315, 360 sh, 385; $AlCl_3$ 280 sh, 300, 355; $AlCl_3$–HCl 285 sh, 300, 350; NaOAc 255 sh, 275, 390; NaOAc–H_3BO_3 270, 335. ^{1}H NMR (as TMSi ether): *δ*7.7 (*d*, $J_{5',6'}$ = 9 Hz, H-2′,6′), 6.9 (*d*, $J_{3',5'}$ = 9 Hz, H-3′,5′), 6.6 (*s*, H-8), 6.5 (*s*, H-3), 3.7 (*s*, 6-Me).

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