and 15% HOAc. Individual spots or complexes of compounds were eluted from sheets and further purified using cellulose and polyamide TLC and Sephadex LH-20 columns with standard solvent systems [9]. Compound identities were determined by standard techniques using R_f values, colour reactions, UV spectrophotometry with diagnostic reagents, and co-chromatography with known samples [9, 10]. Sugars of *O*-glycosides were identified by enzymatic hydrolysis with β-glucosidase and acid hydrolysis followed by circular co-chromatography with commercial standards [11].

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QUERCETAGETIN 6,7,4′-TRIMETHYL ETHER AND 3-SULPHATE FROM *DECACHAETA HAENKEANA*

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Key Word Index—*Decachaeta haenkeana*; Compositae; Eupatorieae; 6-methoxyflavonols; flavonol sulphate.

Abstract—Four 6-methoxylated flavonols, including a new quercetagetin derivative and its 3-potassium sulphate salt, were isolated from the aerial parts of *Decachaeta haenkeana*.

INTRODUCTION

As part of our continuing chemotaxonomic study of the tribe Eupatorieae, we here report the isolation and characterization of four flavonols, including a new flavonol and its 3-*O*-sulphate, from the aerial parts of *Decachaeta haenkeana* DC., the type species of the genus. Although *Decachaeta* has been treated as monotypic by earlier authors [1], a revision by King and Robinson [2, 3] expanded the genus by including six additional species from *Ophryosporous* and *Eupatorium*. These authors maintain *D. haenkeana* in a separate subgenus (subgenus *Decachaeta*), while combining the six new species in a second subgenus, *Polydenia*. Preliminary chemical studies have lent some support to this distinction. The three species studies from subgenus *Polydenia*, *D. thieleana* [4, 5], *D. ovatifolia* [6] and *D. scabrella* [Miski *et al.*, unpublished], all contain sesquiterpene lactones mainly of the guaianolide type, although 7α-hydroxy lactones of several skeletal types are found in *D. ovatifolia* [6]. In contrast, we were unable to isolate any sesquiterpene lactones from *D. haenkeana*.

RESULTS AND DISCUSSION

The dichloromethane extract of *D. haenkeana* afforded one new as well as one known flavonol aglycone. The new flavonol aglycone was established as quercetagetin 6,3′,4′-trimethyl ether (**1**) by the following spectral data. When the compound was viewed on paper under UV light it exhibited a faint yellow colour with and without ammonia which did not change when sprayed with NA reagent.

These colour reactions suggested free 3- and 5-hydroxyl groups and the absence of a free *ortho* 3′,4′-dihydroxyl system. In the UV spectrum of **1** (MeOH) a shoulder at 270 nm together with Band II at 258 nm indicated the presence of 3′,4′-oxygenation in the B-ring. The presence of Band III at 340 nm in the sodium methoxide spectrum and a smaller bathochromic shift of Band I in the sodium acetate spectrum relative to Band I in the sodium methoxide spectrum supported the presence of a 7-hydroxyl function. Decreased intensity of Band I in the sodium methoxide spectrum relative to the methanol spectrum (without decomposition) indicated the compound lacked a free 4′-hydroxyl [7]. The boric acid, aluminium chloride and aluminium chloride + hydrochloric acid spectra again indicated the absence of an *o*-dihydroxyl system in **1**. The ^{1}H NMR spectrum of **1** (in CCl_4 as the TMSi ether) exhibited three methoxyl signals at δ3.92, 3.90 and 3.82; the remaining signals were typical for a trisubstituted A-ring and 3′,4′-disubstituted B-ring flavonol pattern (see Experimental). The ^{1}H NMR spectrum of **1** in benzene-d_6 exhibited a *ca* Δ0.50 ppm upfield shift of two methoxyl signals and Δ0.03 ppm for one methoxyl signal. The MS of **1** displayed an $[M]^+$ ion at *m/z* 360, $[A_1 - Me]^+$ at *m/z* 167 and $[B_2]^+$ at *m/z* 165. Combination of UV, ^{1}H NMR and MS data clearly indicated that two of these methoxyl groups were located at the 3′ and 4′ positions; thus, the third methoxyl group must be at C-6 or C-8. Complete methylation of **1** afforded **2**, the hexamethyl derivative of **1**. Spectral data and direct comparison with an authentic sample established that **2** was identical to quercetagetin hexamethyl ether. Therefore, **1** is quercetagetin 6,3′,4′-trimethyl ether.

Another compound from the same extract was identified from its spectral properties and by direct comparison with an authentic sample as spinacetin (quercetagetin 6,3′-dimethyl ether) (**3**) [8].

The second novel compound precipitated out of the water layer during ethyl acetate extraction. Recrystallization from methanol afforded pure **4**. That **4** was the 3-sulphate analogue of **1** was clear from the following evidence. First, **4** yielded **1** upon treatment with dilute HCl. Further, its electrophoretic mobility was in accord with a monosulphate [9, 10]. Also, the purple colour of **4** when viewed on paper under UV light with and without NH_3 established 3-*O*-substitution. Finally, in the FAB-MS of **4** the $[M + H]^+$ ion observed at *m/z* 479 ($C_{18}H_{15}O_{11}SK + H$), confirmed the presence of a sulphate moiety with a K^+ counter ion. Interestingly, an $[M + K]^+$ (i.e., two K^+ ions present) fragment at *m/z* 517 was also found in the spectrum.

Compound **5** obtained from the ethyl acetate extract was determined to be the 3-glucoside of eupatolitin (quercetagetin 6,7-dimethyl ether) [8, 11] by its spectral properties, hydrolysis and comparison with an authentic sample.

Our results indicate *D. haenkeana* is chemically distinct from the other species currently placed in the genus, supporting its assignment to the monotypic subgenus *Decachaeta*. The occurrence of a sulphated flavonol is particularly interesting since an electrophoretic survey of extracts of all *Decachaeta* species (see Experimental) indicated that sulphated flavonoids are absent from the subgenus *Polydenia*, but are readily detectable in crude extracts of *D. haenkeana*. So far, other studies have shown sulphated flavonoids are present in a few other members of the Eupatorieae, notably species of *Brickellia* [11, 12] and *Hofmeisteria* [Norris, J., personal communication].

EXPERIMENTAL

Plant material. Aerial parts of *D. haenkeana* were collected 15 miles S of Uruapan, Michoacan, Mexico on the road to Playa Azul by John Norris and Jonathan Gershenzon. A voucher specimen (J. N. no. 127) is on deposit at the University of Texas Herbarium. For the electrophoretic survey of *Decachaeta* one or two leaves were removed from each of the following herbarium specimens and extracted in 80% MeOH: *D. haenkeana* (Cronquist no. 9752), *D. scabrella* (McVaugh no. 21278), *D. ovatifolia* (Mexia no. 8805), *D. incompta* (Castillo no. 3011), *D. peronata* (Turner no. 15098), *D. thieleana* (Croat no. 13506) and *D. ovandensis* (Breedlove no. 41815).

General techniques. Mps are uncorr. CC employed polycar AT, microcrystalline cellulose (Avicel) and Sephadex LH-20. Precoated microcrystalline cellulose plates were used for TLC. Electrophoresis were carried out on Whatman 3 MM paper in a pH 1.9 buffer (HCO_2H–HOAc–H_2O, 33 : 147 : 1820). The solvent systems for TLC were: TBA (*t*-BuOH–HOAc–H_2O, 3 : 1 : 1), *n*-BAW, upper layer (*n*-BuOH–HOAc–H_2O, 4 : 1 : 5), 15% and 40% HOAc. Visualization of the flavonoids on TLC plates was realized either by UV light + NH_3 or by spraying with NA (Naturstoffreagenz-A) in MeOH.

Isolation and characterization of flavonoids. Ground, dried leaves (1.8 kg) were extracted with % 85 aq. MeOH (6 l. × 2) and % 50 aq. MeOH (3 l. × 1). Combined extracts were evaporated *in vacuo* until only H_2O remained. The conc. extract was partitioned sequentially against *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH. The conc. CH_2Cl_2 extract (2.3 g) was dissolved in toluene and partitioned with 60% aq. MeOH to remove chlorophyll and other resinous material from the crude flavonoid mixture. The conc. aq. MeOH extract was then re-extracted with CH_2Cl_2, dried with dry $MgSO_4$ and evaporated *in vacuo*, to yield 1.1 g of crude extract. This extract, when chromatographed over Sephadex LH-20 packed in MeOH–CH_2Cl_2 (75 : 25), yielded **1** (32 mg) and **3** (21 mg).

Quercetagetin 6,3′,4′-trimethyl ether (**1**). Yellow needles from MeOH mp 236–238°. R_f values, TBA; 0.76, 15% HOAc; 0.06. UV λ_{max}^{MeOH} nm: 365, 344 (sh), 300 (sh), 270 (sh), 258; NaOMe, 417, 340 (sh), 268, 235; $AlCl_3$, 425, 370 (sh), 300 (sh), 265; $AlCl_3$ + HCl, 422, 370 (sh), 265; NaOAc, 420 (sh), 378, 340 (sh), 300 (sh), 272 (sh), 258; NaOAc + H_3BO_3, 365, 340 (sh), 300 (sh), 270 (sh), 257. ^{1}H NMR (as TMSi ether, 90 MHz, CCl_4): δ6.4 (*s*, H-8), 6.88 (*d*, $J_{5',3'}$ = 9 Hz, H-5′), 7.6 (*d*, $J_{2',6'}$ = 2 Hz, H-2′), 7.7 (*dd*, H-6′), 3.82 (*s*, 4′-OMe*), 3.9 (*s*, 3′-OMe*), 3.92 (*s*, 6-OMe) *interchangeable; (C_6D_6): δ6.11 (*s*, H-8), 6.72 (*d*, H-5′), 7.9 (*dd*, H-6′), 7.98 (*d*, H-2′), 3.29 (*s*, 4′-OMe†), 3.37 (*s*, 3′-OMe†), 3.89 (*s*, 6-OMe) †interchangeable. EIMS (direct probe 70 eV) *m/z* (rel. int.): 360 $[M]^+$ (100), 345 $[M - Me]^+$ (13.2), 342 $[M - H_2O]^+$ (42.3), 332 $[M - CO]^+$ (16), 327 $[M - Me - H_2O]^+$ (15.7), 317 $[M - Me - CO]^+$ (97.5), 299 $[M - Me - CO - H_2O]^+$ (26.1), 167 $[A_1 - Me]^+$ (12.8), 165 $[B_2]^+$ (9.8), 151 $[B_2 - Me + H]^+$ (14).

Complete methylation of **1** *and isolation of* **4**. Compound **1** (10 mg) was refluxed in dry Me_2CO with MeI and K_2CO_3 for 24 hr. After the usual work-up 13 mg of **2** was obtained. During partitioning against EtOAc a brownish solid precipitated from the water layer. After filtration and several recrystallizations from MeOH the ppt afforded **4**, quercetagetin 6,3′,4′-trimethyl ether 3-potassium sulphate salt, as light orange coloured prisms mp 232–233°, dec. (230 mg). R_f values; TBA, 0.54, % 15 HOAc, 0.67. UV λ_{max}^{MeOH} nm: 349, 272, 257; NaOMe, 369, 320, 300 (sh), 278; $AlCl_3$, 380, 280, 270; $AlCl_3$ + HCl, 370, 295 (sh), 281, 266; NaOAc, 420 (sh), 350, 275, 260 (sh); NaOAc + H_3BO_3, 350, 270 (sh), 257. ^{1}H NMR

spectrum and EIMS identical with **1**. FAB-MS *m/z* (rel. int.): 517 $[M+K]^+$ (8.1), 479 $[M+H]^+$ (10.5), 441 $[M-K+2H]^+$ (54), 361 $[Aglyc+H]^+$ (21), 167 $[A_1-Me]^+$ (11.8), 165 $[B_2]^+$ (8), 151 $[B_2-Me+H]^+$ (8.4).

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A FURTHER QUINAZOLINE ALKALOID FROM *ADHATODA VASICA**

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Key Word Index—*Adhatoda vasica*; Acanthaceae; quinazoline alkaloid; 1,2,3,9-tetrahydro-5-methoxypyrrolo[2,1-*b*]quinazoline-3-ol.

Abstract—A new quinazoline alkaloid isolated from the leaves of *Adhatoda vasica* has been identified as 1,2,3,9-tetrahydro-5-methoxypyrrolo[2,1-*b*]quinazoline-3-ol.

INTRODUCTION

Adhatoda vasica (Acanthaceae) is known to furnish quinazoline alkaloids [1]. Vasicine (**1**) and vasicinone (**2**), the two alkaloids of the plant are remarkable in bioactivity [2–5]. In the course of our investigations on the biologically active compounds related to vasicine and vasicinone, we undertook further chemical examination of the leaves of the plant. The present investigation revealed the presence of the quinazoline alkaloid (**3**) which hitherto has not been reported as a constituent of any natural material.

*Part 2 in the series "Vasicine and Related Compounds". For Part 1 see Chowdhury, B. K., Afolabi, E. O., Sokomba, E. N. and Osuide, G. (1985) *Indian J. Chem.* (in press).

RESULTS AND DISCUSSION

The alkaloid **3**, $C_{12}H_{14}N_2O_2$ ($[M]^+$ *m/z* 218), mp 224–225° was optically inactive and was found to be homogeneous by TLC and mass spectrometry. The UV spectrum of the alkaloid showed a maximum at 307 (log ε3.85) nm. The IR spectrum (KBr) showed absorption bands at 3470 (OH), 1630 (>C=N–), 1605, 1500 (aromatic residue), 1250 (aromatic ether) and 845 cm^{-1} (substituted benzene derivative). The ^1H NMR spectrum showed signals at δ6.60–7.0 (*m*, 3H, aromatic protons), 4.71 (*t*, 1H, C-3), 4.50 (*s*, 2H, C-9), 3.80 (*s*, 3H, aromatic methoxyl), 3.21 (*m*, 2H, C-1) and 2.18 (*m*, 2H, C-2). The mass spectrum of **3** showed an $[M]^+$ at *m/z* 218, the base peak appearing at *m/z* 217 $[M-1]^+$ due to the formation of the quinazolinium ion (**4**). The mass spectrum also revealed ions at *m/z* 203 $[M-15]^+$ and 199 $[M-1-18]^+$ which supported the presence of methoxyl and hydroxyl groups.

All these data and a direct comparison of the ^1H NMR