Pergamon

PII: S0031-9422(97)00370-1

Phytochemistry, Vol. 46, No. 5, pp. 935–941, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

POLYPHENOLIC METABOLITES OF *EPILOBIUM HIRSUTUM*

HEBA H. BARAKAT, SAHAR A. M. HUSSEIN, MOHAMED S. MARZOUK, IRMGARD MERFORT*, MICHAEL LINSCHEID† and MAHMOUD A. M. NAWWAR‡

National Research Centre, Dokki, Cairo, Egypt; * Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätstrasse 1, 40225 Düsseldorf, Germany; † Institut für Spektrochemie, Bunsen-Kirchhoffstrasse 11, 44013 Dortmund, Germany

(*Received in revised form* 3 *March* 1997)

Key Word Index—*Epilobium hirsutum*; Onagraceae; ellagitannins; gallotannins; flavonoids; 2-*O*-galloyl 3-*O*-valoneoyl dilactone-(α/β)-$^4C_1$-glucopyranose; 1′-monodecarboxyvaloneic acid dilactone; valoneic dilactone dioxine; NMR; HPLC quality assessment of the extract.

Abstract—Evaluation of the constitutive polyphenolics of the whole plant extract of *Epilobium hirsutum* was carried out by reverse-phase HPLC. The unique ellagitannins, 2-*O*-galloyl 3-*O*-valoneoyl dilactone-(α/β)-$^4C_1$-glucopyranose, 1′-monodecarboxyvaloneic acid dilactone and valoneic dilactone dioxine were isolated and characterized. The known polyphenolics, gallic, protocatechuic, ellagic, valoneic dilactone and *p*-coumaric acids, methyl gallate, *p*-methoxy gallic acid methyl ester, 6-*O*-galloylglucose, 1,6-di-*O*-galloylglucose, 2,3-di-*O*-galloylglucose, 1,2,6-tri-*O*-galloylglucose and the 3-*O*-glucuronides, 3-*O*-arabinosides and 3-*O*-rhamnosides of kaempferol, quercetin and myricetin, their free aglycones 3-*O*-galactoside and quercetin and myricetin were also identified. The structures were established by conventional methods of analysis and confirmed by ^1H, ^{13}C NMR and negative ESI-mass spectrometry. 2D-long range selective proton decoupling and chemical shift correlation NMR experiments were applied for the new polyphenolics. The HPLC phenolic profile was used for the quality assessment of the *E. hirsutum* extract for medicinal purposes and also as a fingerprint for the authentication of the plant material. © 1997 Elsevier Science Ltd

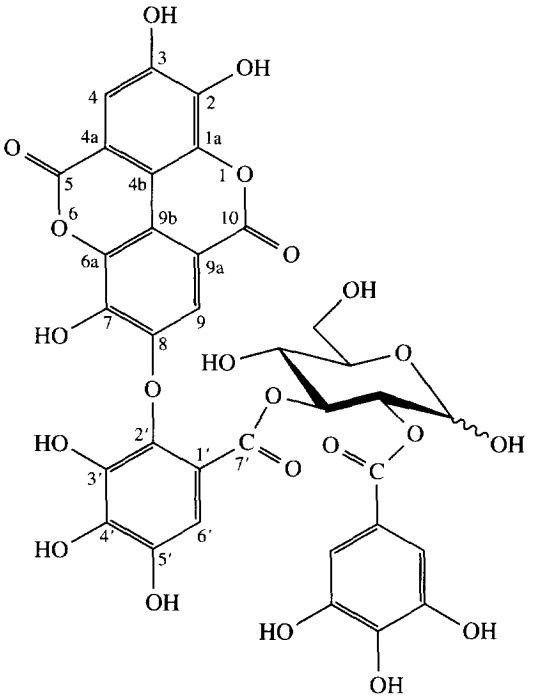
INTRODUCTION

Epilobium hirsutum L. is a softly hairy herb, which grows in the marshy ground near the Mediterranean coastal-strip of Egypt [1]. It is used in Egyptian and Europian folkmedicine for treating inflammation, adenoma and prostate tumours [2, 3]. In a previous phytochemical study, we have reported the establishment of optimum conditions for assaying the complex mixture of ellagi-, gallo-tannins and flavonoids, contained in the aqueous ethanolic *Epilobium hirsutum* whole plant extract by the reverse-phase HPLC/−ve ESI-mass spectrometric technique. We also reported the isolation and structural elucidation of the new ellagitannin, valoneic acidamide dilactone (epilobamide-A) [4], together with valoneic acid dilactone from this extract. In the present study, the polyphenolic profile was evaluated using reverse-phase HPLC and the isolation and structural elucidation of three new natural polyphenolics: 2-*O*-galloyl 3-*O*-valoneoyl dilactone-(α/β)-$^4C_1$-glucopyranose (**14**), 1′-monodecarboxyvaloneic acid dilactone (**28**) and valoneic dilactone dioxine (**29**) are described together with 26 known compounds (**1–13** and **15–27**). We also propose the use of the HPLC phenolic profile as a convenient means for the quality assessment of *E. hirsutum* in herbal medicine and for the authentication of different batches of the dried herb.

‡Author to whom correspondences should be addressed.

RESULTS AND DISCUSSION

The aqueous ethanolic whole plant extract of *E. hirsutum* was shown by 2D-PC screening to contain a complicated polyphenolic mixture of ellagitannins (purple colour with nitrous acid test [5]), gallotannins (rosy red colour with KIO_3 test [6]) and flavonoids (colour properties under UV light). This complex mixture was analysed by gradient reversed-phase HPLC analysis, which revealed the presence of at least 30 components, of which 29 **1–29** were isolated and purified by standard methods (CC and prep. PC). Known compounds (**1–13** and **15–27**) gave chromatographic, UV spectral, −ve ESI-MS, hydrolytic and ^1H NMR data typical for gallic acid **1** [7], methyl gallate **2** [7], 6-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose **3** [8], *p*-methoxygallic acid methyl ester **4** [7], *p*-coumaric acid **5** [9], 1,6-di-*O*-galloyl-β-$^4C_1$-glucopyranose **6** [6], pro-



14

tocatechuic acid **7** [10], 2,3-di-*O*-galloyl-(α/β)-$^4C_1$-glucopyranose **8** [7], quercetin 3-glucuronide **9** [11], myricetin 3-rhamnoside **10** [12], quercetin 3-rhamnoside **11** [12], kaempferol 3-rhamnoside **12** [12], 1,2,6-tri-*O*-galloyl-β-$^4C_1$-glucopyranose **13** [13], kaempferol 3-glucuronide **15** [11], myricetin 3-galactoside **16** [12], myricetin 3α-arabinopyranoside **17** [14], quercetin 3-galactoside **18** [15], quercetin 3-α-arabinopyranoside **19** [14], kaempferol 3-α-arabinopyranoside **20** [14], myricetin 3-glucuronide **21** [4], kaempferol **22**, quercetin **23**, myricetin **24**, valoneic acid dilactone **25** [4], ellagic acid* **26** [13] and valoneic acidamide dilactone (epilobamide-A) **27** [4].

The new compound **14**, a white amorphous powder, was found to possess chromatographic properties (Table 1), colour reactions (dark purple spot on PC under short UV, intense blue with $FeCl_3$, pink with KIO_3 specific for gallotannins and intense orange with HNO_2 specific for ellagitannins) and UV spectra data (Table 1) consistent with an ellagitannin bearing galloyl moiety/ies. It exhibited an M_r of 784, corresponding to a molecular ion $[M-H]^-$ at m/z 783 and an R_t of 19.72 min, on reverse-phase HPLC analysis. Direct measurement of the ESI-mass spectrum at collision induced dissociation voltage (CID) led to the formation of fragment ions at m/z 481 (M_r† 482), 469 (M_r† 470), 451 (M_r† 452), 301 (M_r† 302) and 169 (M_r† 170), a pattern of fragmentation which could be interpreted in terms of the proposed galloyl-valoneoyl dilactone-glucose structure for **14**. This view was supported by complete acid hydrolysis which yielded glucose (CoPC), valoneic acid dilactone and gallic acid (CoPC, UV, ^1H and ^{13}C NMR data [4]). On controlled acid hydrolysis **14** gave, among other hydrolysis products, an intermediate (**14a**), which after purification showed chromatographic properties and colour reactions typical for an ellagitannin lacking galloyl moieties (intense orange nitrous acid test, negative response towards KIO_3 and intense $FeCl_3$ blue colour). Compound **14a** showed on −ve ESI-mass spectrum an $[M-H]^-$ ion peak at m/z 631 and two fragment ions (when measured at CID voltage) at m/z 301 and 299, thus proving an M_r of 632 for **14a** and suggesting a valoneoyl dilactone glucopyranose structure which was confirmed by complete acid hydrolysis of **14a** to yield glucose and valoneic acid dilactone (CoPC).

The ^1H NMR spectrum of **14** revealed two distinct patterns of proton resonances belonging to substituted α- and β-glucose anomers. Each pattern was found to contain well separated resonances of the seven-spin system belonging to a distinct glucose anomer, thus proving absence of substitution at the glucose anomeric hydroxyl group. The presence, in this spectrum, of the diagnostic H-2_α resonance downfield at δ 4.88 ppm (dd, $J = 8$ and 2.5 Hz) proved acylation at this hydroxyl group. Measurement of the COSY spectrum for **14** confirmed this finding and proved additional acylation at the 3-hydroxyl glucose, whose proton was found resonating downfield at δ 5.68 (t, $J = 8$ Hz, H-3_α) and at 5.18 (t, $J = 9$ Hz, H-3_β), while the resonance of the H-2_β proton was found to resonate at δ 4.98 (*dd*, $J = 8$ and 9 Hz). The location of other glucose proton resonances, comparatively upfield proved the absence of acylation at all the other glucose hydroxyl groups except nos 2 and 3. These and the above given data (specially results of controlled acid hydrolysis) would suggest a 2-*O*-galloyl-3-*O*-valoneoyl dilactone-(α/β)-$^4C_1$-glucose structure for **14**. A reversed substitution as in the case of the known compound 2-*O*-valoneoyl dilactone-3-*O*-galloyl-(α/β)-$^4C_1$-glucose (oenothein C) [16], would produce on partial hydrolysis a 3-*O*-galloylglucose as it is well known that an ester linkage at the glucose 2-OH is more labile and easily hydrolysed than the ester linkage at position no. 3. This was confirmed by the measurement of a ^1H-^{13}C long range shift correlation spectrum of **14**, (Fig. 1) which revealed two cross peaks correlating the galloyl carbonyl carbon resonances in each of the α- and β-anomers to the resonances of the glucose H-2 proton resonances. Cross peaks correlating the valoneoyl dilactone carbonyl carbon signals in both anomers to the resonances of the H-3 glucose protons were also recognized, thus

*The ^{13}C NMR data published for ellagic acid, by the author in ref. [13] contain a wrong δ value for the resonance of the two equivalent atoms C-3 and C-8. The correct value is 148 ppm.

† Fragment molecular weight.

Table 1. Chromatographic, UV and −ve ESI-MS data for **14**, **28** and **29**

Compound	Chromatographic properties R_t (min.)	R_f (× 100) H_2O	HOAc-60	BAW	UV spectral data λ_{max}^{MeOH} (nm)	−ve ESI-MS [M−H]⁻ *m/z*	CID data *m/z*
14	19.72	—	52.6	58.0	362, 347 and 300 sh., 257, 217	783	481, 469, 45 301, 299, 16
14a	18.69	—	61.9	32.7	362, 347 and 300 sh., 257, 217	631	301, 299
Valoneic acid dilactone (**25**)	21.84	—	48.7	44.3	362, 347 and 300 sh., 256, 215	469	425, 301, 30 299
Gallic acid	13.51	53.0	—	78.0	272	169	125
28	27.28	—	44.9	42.6	361, 347 and 300 sh., 256, 215	425	301, 300, 29
29	—	0.0	1.80	5.50	360, 347 and 300 sh., 254	423	301, 300, 29

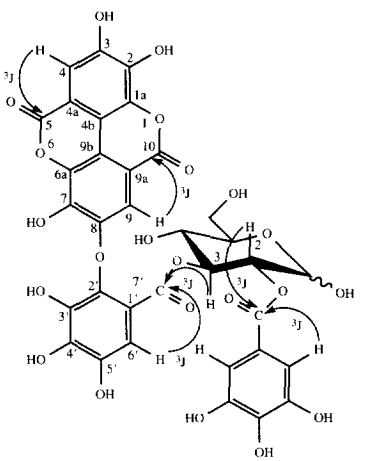
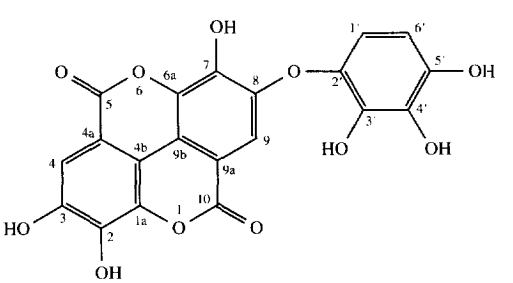


Fig. 1.

confirming the structure of **14** to be 2-*O*-galloyl-3-*O*-valoneoyl dilactone-(α/β)-$^4C_1$-glucose. The ^{13}C NMR data of **14** further confirmed its achieved structure. The α- and β-anomers are recognized, in the recorded spectrum from the resonances at δ ppm 90.6 and 95.6, respectively. The acylation by galloyl and valoneoyl dilactone moieties, of the glucose OH groups at positions 2 and 3 follows from the upfield shift of the resonances of C-1 and C-4 compared to the corresponding resonances in free D-glucopyranose [7]. These β-effects are in agreement with those reported for similarly substituted glucopyranose [7]. The upfield shifts of the resonances of C-2 and C-3 are caused by both α- and β-effects which brought these resonances to δ ppm 73.9 (C-2_β), 73.1 (C-2_α), 77.1 (C-3_β) and 74.1 (C-3_α). The dilactone structure of the valoneoyl moiety was evidenced by the carbonyl carbon resonances at δ ppm 159.8, 159.71 of C-5 and 159.6, 159.5 of C-10 in both anomers. Presence of only one galloyl moiety and one valoneoyl dilactone moiety in **14** follows from the two galloyl C=O carbonyl carbon resonances (one for each anomer) and the two valoneoyl dilactone C=O carbonyl carbon resonances (one for each anomer) at δ ppm 167.14 and 167.04 (galloyl C=O) and at δ ppm 164.47 and 164.24 (valoneoyl dilactone carboxyl C=O). Assignment of the remaining carbon resonances were aided by comparison with the previously reported data of analogous compounds [4, 16, 17]. Furthermore, the measured δ-values of the glucose carbon resonances confirmed that the sugar core exists in the pyranose form. Consequently, **14** is 2-*O*-galloyl-3-*O*-valoneoyl dilactone-(α/β)-$^4C_1$-glucopyranose, which has not been reported previously in nature.

The second new polyphenolic (**28**) was isolated as a white amorphous powder which exhibited chromatographic properties and UV spectral data (Table 1) similar to those of valoneic acid dilactone [4]. It resisted normal acid (2 N aqueous HCl, 100°, 3 hr) and alkaline hydrolysis (1 N aqueous KOH, 100°, 1 hr), but yielded pyrogallol and ellagic acid (CoPC, UV and ^1H NMR spectral data) on alkali-fission (40% aqueous KOH, 100°, 1/2 hr). The −ve ESI-mass spectrum of **28** revealed a molecular ion peak [M-H]⁻ at *m/z* 425, corresponding to an M_r of 426. Measurement under CID conditions afforded two fragment symmetric ions [M-125]⁻ and [M-126]⁻, attributable to a monodehydroellagic acid moiety. The IR spectrum of **28** showed strong absorptions at ν_{max} 821.9, 916.0,



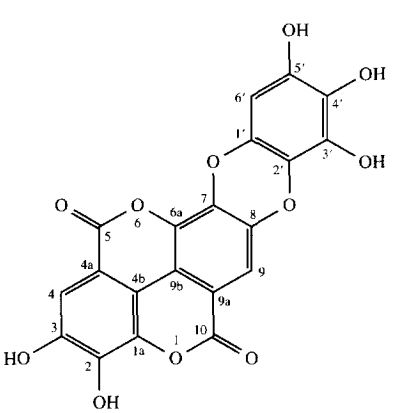
28

1'-Monodecarboxyvaloneic acid dilactone

1062.8, 1103.1, 1239.2, 1289.4, 1343.7, 1738.2 and 3417.1 cm^{-1}, consistent with C—O—C symmetrical stretching in lactone ring, aromatic-H, symmetrical stretching of bi-aryl ether, C—O—C assymetrical stretching in lactone ring, asymmetrical stretching of bi-aryl ether, C—O stretching, O—H deformation, C=O stretching and O—H stretching, respectively. The chemical and spectral data given above suggested a monodecarboxy-valoneic acid dilactone structure for **28**.

The ^1H NMR of analysis **28** lent support for this view. Thus, two aromatic proton resonances were found at δ ppm 7.5 and 7.3 as sharp singlets, assignable to H-4 and H-9 in the monodehydroellagic acid moiety (see formula) and two doublets at δ ppm 6.5 (*d*, $J = 9$ Hz) and 6.4 (*d*, $J = 9$ Hz) which were assigned to the two o-coupled H-1′ and H-6′ protons in the oxypyrogallol moiety of **28**. The structure of **28** was confirmed by ^{13}C NMR spectral analysis which showed 20 distinct carbon resonances, the assignment of which was aided by comparison with the ^{13}C NMR data of valoneic acid dilactone (see experimental) and of 1′-monodecarboxydehydrodigallic acid [18]. The two most downfield resonances at δ ppm 161.31 and 161.25 were assigned to the non-equivalent lactone carbonyl carbons C-5 and C-10, respectively. The absence of any signal more downfield than 161.31 ppm confirms the absence of carboxylic carbons as expected. Carbon resonances at δ ppm 111.93, 111.77, 112.85 and 107.35 were attributed to the protonated carbons C-4, C-9, C-1′ and C-6′, respectively. These assignments together with those of the remaining carbon resonances were confirmed by measurement of gated decoupled and HETCOR spectra. Thus the structure of **28** is 1′-decarboxy valoneic acid dilactone which represents to the best of our knowledge a new natural polyphenolic metabolite.

The third new polyphenolic **29** was separated as a yellow amorphous powder which gave a blue $FeCl_3$ test, but showed no response towards nitrous acid or KIO_3 tests. It was recovered unchanged after refluxing at 100° for 3 hr with aqueous 2 N HCl, but split into ellagic acid and pyrogallol on alkali-fission, thus proving that both of the released moieties are incorporated in its molecule, most probably connected through an ether linkage. −ve ESI-mass spectral analysis of **29** afforded a molecular ion peak $[M-H]^-$ at *m/z* 423, corresponding to an M_r of 424 and fragment ions at *m/z* 300 and 299 which proved the presence of an ellagic acid moiety in the molecule. These data together with the UV absorption behaviour suggested that **29** originated from 1′-monodecarboxy valoneic acid dilactone through the loss of two hydrogen atoms. The ^1H NMR spectral data of **29** was in accordance with the proposed structure. The two diagnostic ellagic acid proton resonances H-4 and H-9 (see formula) appeared as sharp singlets at δ ppm 7.4 and 7.28, respectively and an upfield aromatic proton singlet at δ ppm 6.12 was assigned to a proton whose α-carbon is connected from both sides with oxygenated β-carbons (as C-8 in case of 7-hydroxy flavonoids [12]), i.e. to the H-6′ proton of the pyrogallol moiety in **29**. The presence of only one pyrogallol proton resonance in this spectrum is best interpreted in terms of a dioxine structure in which two ether bridges connect the pyrogallol moiety to the ellagic acid moiety resulting in a valoneic dilactone dioxine.



29
Valoneic dilactone dioxine

Confirmation of the dioxine structure of **29** was obtained by ^{13}C NMR spectral analysis, which revealed 20 individual carbon resonances, among which the most upfield resonance at δ ppm 94.1 was attributed to C-6′ in the pyrogallol moiety of **29**. This recognizable upfield shift, in comparison to the resonance of the corresponding carbon in the molecule of 1′-monodecarboxyvaloneic acid dilactone (**28**), (see Experimental) is clearly due to the formation of the second C_7-O-C_1, ether bridge in **29**. Chemical shift values of the remaining carbon resonances in this spectrum were in close agreement with the achieved structure of **29** as valoneic dilactone dioxine, which represents another new natural polyphenolic.

The comprehensive phytochemical studies of 39 other *Epilobium* species has shown clear differences in the two dimensional chromatograms/flavonoid profiles between them [2, 19, 20], therefore, we suggest that the HPLC phenolic profile could prove useful in the quality assessment of *E. hirsutum* extract used in herbal medicine [2, 3], and also allows the accurate authentication of the plant material. Quantitative HPLC analysis of eight different plant samples, collected heterogeneously, during October 1995, from different locations in the Egyptian marshes close to the Mediterranean coast, extending from Port-Saeid to Rafah, produced very similar HPLC chromatograms.

EXPERIMENTAL

^1H NMR: 400 MHz. ^1H NMR resonances were measured relative to TMS and ^{13}C NMR resonances in DMSO-d_6 and converted to TMS scale by adding

39.5. Typical conditions: spectral width = 6000 for ^1H and 22000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. −ve ESI-MS spectra were measured on an SSQ Finnigan MAT 4600 quadrupol mass spectrometer equipped with a home-built ES-ion source (ISAS Dortmund). A soln of the compound was directly injected by a syringe Harvard infusion pump, into the sprayer of the ES source. MeOH as sheath flow (5 μL min^{-1}) was also fed into the sprayer through a stainless steel capillary, while SF_6 with a flow *ca* 9 ml min^{-1}, was fed to the spray chamber sample through the outer capillary, to prevent plasma formation during the production of negative ions. In the ion source the desolvation capillary (i.d.: *ca* 0.5 and l = 145 mm) was heated to appropriate temp for declustering and desolvation (T = 200–280°) depending on the nature of the sample. The ESI voltage is 3 KV above the acceleration potential of the spectrometer (*ca* 100 V). Desolvation capillary/first skimmer voltage is about 80 V, raised up to 500 V for initiating collision induced dissociations (CID). Reverse-phase HPLC was performed using a Gynkotek pump, model 480, Germering, Germany; a Rheodyne injection valve model 7125, Eppelheim, Germany; a spectro-monitor UV detector, model 1204A-LDC, Darmstadt, Germany and a D-2000 chromato integrator, Merck-Hitachi, Düsseldorf, Germany. Samples were injected as solns. in MeOH–H_2O (4:1). Sepn was carried out on a Lichrospher RP-18E (250 × 4 mm, 5 μm) column, using solvent system: (A) H_2O–H_3PO_4 (999:1), (B) MeOH at a flow rate of 0.5 ml min^{-1}, injection vol. 20 μl and UV detection at 280 nm. Time programme for gradient elution: min/% of (B): 0/0, 30/100, 35/100 and 100/0. PC was carried out on Whatman no. 1 paper, using solvent systems: (1) H_2O; (2) 6% HOAc-6; (3) 15% HOAc; (4) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer); (5) C_6H_6-*n*-BuOH–H_2O–pyridine (1:5:3:3, top layer). Solvents 2, 3 and 4 were used for prep. PC on Whatman no. 3 mm paper, solvents 4 and 5 for sugar analysis.

Plant material. Fresh herbs of *Epilobium hirsutum* L. were collected from the marshy habitats around 'Port-Saied', Egypt, in October 1994 and authenticated by Dr L. Boulos, Professor of Botany, NRC, Cairo, Egypt. A voucher specimen has been deposited at the herbarium of the NRC.

Isolation and identification. Herbs of *E. hirsutum*, dried in the shade in an air-draft, were comminuted to powder and exhaustively extracted with EtOH–H_2O (4:1). The concd. extract was applied to a polyamide 6S CC (Riedel-De Häen AG, Seelze Hannover, Germany) and eluted by H_2O followed by H_2O–EtOH mixts of decreasing polarity to yield 13 major frs (I–XIII). Compounds **1** (148 mg) and **2** (84 mg) were isolated from fr. II by CC over sephadex LH-20 using EtOH as eluant and **3** from fr. III by CC on sephadex LH-20 using *n*-BuOH saturated with H_2O for elution, followed by repeated pptn of the desorbed material from Me_2CO by Et_2O (22 mg). Compounds **4** (45 mg) and **5** (58 mg) were obtained from fr. IV by prep. PC using 6% HOAc as solvent and **6** (69 mg) from fr. V by sephadex LH-20 CC using EtOH for elution and purification by Me_2CO–Et_2O pptn. Compounds **7** (41 mg), **8** (48 mg), **9** (39 mg), **10** (35 mg), **11** (39 mg) and **12** (15 mg) were isolated from fr. VI by sephadex LH-20 CC using EtOH as solvent and repeated prep. PC, **13** (78 mg) from fr. VII by sephadex LH-20 CC using EtOH as solvent and Me_2CO–Et_2O pptn for purification and **15** (35 mg) from fr. VIII by sephadex LH-20 CC using *n*-BuOH–*iso*pr.OH–H_2O, 4:1:5, (top layer) followed by prep. PC using BAW. Compounds **16** and **17** were isolated from fr. IX by sephadex LH-20 CC using EtOH as solvent followed by prep. PC (BAW) and crystallization (41 and 35 mg, respectively), **18** and **19** from fr. X by prep. PC (BAW), (35 mg of **18** and 42 mg of **19**), **20** and **21** from fr. XI by prep. PC (BAW) and compounds (**22**–**27**) by fractional extraction by $CHCl_3$, followed by cellulose CC (Cellulose microcrystalline for CC $[C_6H_{10}O_5]_n$, E. Merck, Darmstadt, Germany) using *n*-BuOH saturated with H_2O as solvent to afford 21 mg of **22**, 33 mg of **23**, 27 mg of **24**. Sephadex LH-20 CC of the residue left after $CHCl_3$ extraction, using EtOH as solvent yielded 185 mg of **25**, 32 mg of **26** and 60 mg of **27**. Compound **14** was pptd from a concd methanolic soln. of fr. VIII and purification of the ppt. by sephadex LH-20 using EtOH as solvent (yield 95 mg). Compounds **28** and **29** were isolated from fr. XIII by sephadex LH-20 using EtOH as solvent to give 65 mg and 31 mg, respectively.

2-O-*Galloyl*-3-O-*valoneoyl dilactone*-(α/β)-$^4C_1$-*glucopyranose* (**14**). R_f*s*, R_t and UV spectral data: Table 1. M_r 784, −ve ESI-MS, $[M-H]^-$ = *m/z* 783, fragment ions under CID conditions: *m/z* 481, 451, 301, 299 and 169. **14** yielded glucose, gallic acid and valoneic acid dilactone (CoPC) on complete acid hydrolysis [55 mg of **14** were refluxed with 20 ml, 2 N aq. HCl, 100°, 2 hr]. Valoneic acid dilactone was filtered off from the cold hydrolysate, and gallic acid extracted by EtOAc from the filtrate. Valoneic acid dilactone: R_f*s*, R_t and UV spectral data: Table 1; ^1H NMR, in DMSO-d_6: δ (ppm) 7.49 (*s*, H-4), 6.93 (*s*, H-9), 6.99 (*s*, H-6′); ^{13}C NMR, in DMSO-d_6: δ (ppm) 166.1 (C-7′), 159.4 (C-10), 159.3 (C-5), 149.6 (C-8), 148.7 (C-3), 143.2 (C-5′), 140.8 (C-7), 139.8 (C-2), 139.7 (C-3′), 139.4 (C-2′), 136.8 ($C\text{-}1_a$), 136.4 ($C\text{-}6_a$), 135.4 (C-4′), 114.8 (C-1′), 114.1 ($C\text{-}9_b$), 112.1 ($C\text{-}4_b$), 110.6 (C-4), 108.6 (C-6′), 108.5 (C-9), 108.3 ($C\text{-}4_a$), 107.0 ($C\text{-}9_a$). Gallic acid: R_f*s*, R_t and UV spectral data: Table 1; ^1H NMR, in DMSO-d_6: δ (ppm) 6.98 (s, H-2 and H-6); ^{13}C NMR, in DMSO-d_6: δ (ppm) 120.6 (C-1), 108.6 (C-2 and C-6), 145.5 (C-3 and C-5), 138.1 (C-4), 167.6 (C-7). Controlled acid hydrolysis of **14** [1 N aq. HCl, 100°, 1 hr] yielded an intermediate **14a** which was isolated by prep. PC (BAW). **14a**: R_f*s*, R_t and UV spectral data: Table 1; −ve ESI-MS and CID data: M_r 632, $[M-H]^-$ = *m/z* 631, fragment ions: *m/z* 301 and 299; complete acid hydrolysis yielded valoneic acid dilactone and glucose (CoPC). ^1H NMR of **14**, in

$(CD_3)_2CO$: δ (ppm): α-glucose moiety: 5.68 (*t*, $J = 8$ Hz, H-3), 5.20 (*d*, $J = 2.5$ Hz, H-1), 4.88 (*dd*, $J = 8$ and 2.5 Hz, H-2), 3.91 (*m*, H-5), 3.79 (*t*, $J = 10$ Hz, H-4), 3.73 (*dd*, $J = 12$ and 2.5 Hz, H-6) and 3.69 (*dd*, $J = 12$ and 5 Hz, H-6′); β-glucose moiety: 5.18 (*t*, $J = 9$ Hz, H-3), 4.98 (*dd*, $J = 8$ and 9 Hz, H-2), 4.56 (*d*, $J = 8$ Hz, H-1), 3.91 (*m*, H-5 and H-6′) and 3.72 (*m*, H-4 and H-6); galloyl moiety: 6.94 (*s*, H-2 and H-6, in one anomer), 7.02 (*s*, H-2 and H-6, in the second anomer); valoneoyl dilactone moiety: 7.57, 7.56 (each as *s*, 1H in total, H-4), 7.17, 7.18 (each as *s*, 1H in total, H-6′), 7.05, 7.06 (each as *s*, 1H in total, H-9). ^{13}C NMR of **14**, in $(CD_3)_2CO$: δ (ppm): α-glucose moiety: 90.6 (C-1), 73.1 (C-2), 74.1 (C-3), 69.7 (C-4), 72.5 (C-5) and 62.1 (C-6); β-glucose moiety: 95.6 (C-1), 73.9 (C-2), 77.1 (C-3), 69.7 (C-4), 77.3 (C-5) and 62.1 (C-6); valoneoyl dilactone moiety: 164.5, 164.2 (C-7′ in both anomers), 159.8, 159.7 (C-5), 159.6, 159.5 (C-10), 149.9, 149.8 (C-8 in), 148.9, 148.8 (C-1), 143.7, 143.73 (C-5′), 141.3 (C-7 in both anomers), 140.6, 140.5 (C-4′), 140.2, 140.23 (C-2), 139.7, 139.6 (C-3′), 137.6, 137.4 ($C\text{-}1_a$ and $C\text{-}6_a$ in both anomers), 136.3, 136.0 (C-2′), 115.3 ($C\text{-}9_b$ in both anomers), 114.3, 113.9 (C-1′), 113.4 ($C\text{-}4_b$ in both anomers), 114.4 (C-4 in both anomers), 110.4 (C-6′ in both anomers), 109.3 (C-9 in both anomers), 109.2 ($C\text{-}4_a$ in both anomers), 108.9 ($C\text{-}9_a$ in both anomers). Long range $^1H\text{-}^{13}C$ shift correlation of **14**.

1′-Monodecarboxyvaloneic acid dilactone (**28**). R_f*s*, R_t and UV spectral data: Table 1. Mr 426, −ve ESI-MS, $[M\text{-}H]^- = m/z$ 425, fragment ions under CID conditions: m/z 301, 300 and 299. IR spectral data: ν_{max} cm^{-1}: 576.8, 600.2, 705.8, 737.4, 756.8, 779.9, 820.7, 837.6, 921.7, 967.3, 1011.0, 1183.7, 1195.7, 1228.1, 1366.4, 1409.9, 1462.3, 1493.6, 1528.0, 1586.0, 821.9, 916.0, 1062.8, 1103.1, 1239.2, 1289.4, 1343.7, 1738.2 and 3417.1. On alkali-fission, **28** yielded pyrogallol and ellagic acid (CoPC, UV and ^1H NMR spectral data), [18 mg of **28** were refluxed with 10 ml aq. 40% KOH, 100°, 1/2 hr]. ^1H NMR of **28**, in CD_3OD: δ (ppm) 6.40 (*d*, $J = 9$ Hz, H-6′), 6.50 (*d*, $J = 9$ Hz, H-1′), 7.30 (*s*, H-9) and 7.50 (*s*, H-4). ^{13}C NMR of **28**, in CD_3OD: chemical shifts, multiplicities and coupling constants (Hz): 161.25 *d* (4 Hz, C-10), 161.3 *d* (3 Hz, C-5), 150.7 *d* (3 Hz, C-8), 149.9 *d* (3 Hz, C-3), 144.9 *dd* (11 and 3 Hz, C-5′), 143 *d* (7 Hz, C-7), 141.0 *d* (6 Hz, C-2), 139.7 *d* (8 Hz, C-3′), 137.4 *dd* (11 and 4 Hz, C-2′), 137.8 *br s* ($C\text{-}1_a$), 137.7 *br s* ($C\text{-}6_a$), 136.2 *d* (8 Hz, C-4′), 112.9 *d* (161 Hz, C-1′), 115.8 *d* (7 Hz, $C\text{-}9_b$), 113.5 *d* (7 Hz, $C\text{-}4_b$), 111.9 *d* (166 Hz, C-4), 107.4 *d* (159 Hz, C-6′), 111.8 *d* (165 Hz, C-9), 109.8 *br s* ($C\text{-}4_a$) and 108.5 *br s* ($C\text{-}9_a$).

Valoneic dilactone dioxine (**29**). R_f*s* and UV spectral data: Table 1. M_r 424, −ve ESI-MS, $[M\text{-}H]^- = m/z$ 423, fragment ions under CID conditions: m/z 301, 300 and 299. On alkali-fission, **29** yielded pyrogallol and ellagic acid (CoPC), [12 mg were refluxed with 5 ml aq. 40% KOH, 100°, 1/2 hr]. ^1H NMR of **29**, in DMSO-d_6: δ (ppm) 6.12 (*s*, H-6′), 7.28 (*s*, H-9) and 7.40 (*s*, H-4). ^{13}C NMR of **29**, in DMSO-d_6: δ (ppm) 159.0 (C-10), 158.6 (C-5), 149.6 (C-8), 151.2 (C-3), 142.5 (C-5′), 149.1 (C-7), 141.8 (C-2), 134.2 (C-3′), 123.1 (C-2′), 136.2 ($C\text{-}1_a$), 135.2 ($C\text{-}6_a$), 132.5 (C-1′), 116.2 ($C\text{-}9_b$), 112.1 ($C\text{-}4_b$), 131.28 (C-4′), 112.1 (C-4), 94.1 (C-6′), 110.4 (C-9), 108.2 ($C\text{-}4_a$) and 112.3 ($C\text{-}9_a$).

Plant anthentication. The HPLC flavonoid/phenolic profile of an *E. hirsutum* ethanolic whole plant extract was recorded for 8 different accessions. An accurately weighed specimen from each sample (2.091, 2.087, 2.009, 2.00, 2.060, 2.066, 2.00 and 2.08 g) was extracted by 10 ml EtOH under reflux over a boiling water-bath for 1 hr, filtered while hot and dried under vacuum. The dry material, dissolved in 10 ml MeOH was subjected to reverse-phase HPLC analysis to give almost the same HPLC chromatogram in each case. A methanolic solution of 1′-decarboxyvaloneic acid dilactone **28** (1 mg ml^{-1}) was used as a standard by being added 1:9 proportion to the injected methanolic extract solution (1 ml standard soln. added to 9 ml extract soln.). Compound **28** with an R_t 27.09 min was used as an int. standard.

Acknowledgements—The authors express their deep gratitude to Deutsche Forschungsgemeinschaft for financial support for the project (W-383/6-1). They are also much indebted to Professor J. Buddrus and to Dr J. Lambert (Institut f. Spectrochemie, Dortmund, Germany) for help with the NMR analyses.

REFERENCES

1. Täckholm, V., *Student Flora of Egypt*. Cairo University Press, Egypt, 1974, p. 378.
2. Hiermann, A., *Scientific Pharmacology*, 1983, **51**, 158.
3. Hostettmann, K., *International bioflavonoids Symposium 9th (Hungarian) Bioflavonoids Symposium*, Wien, 1995.
4. Linscheid, M., Marzouk, M. S. A. and Nawwar, M. A. M., *Organic Mass Spectrometry*, submitted.
5. Gupta, R. K., Al-Shafi, S. M., Layden, K. and Haslam, E. *Journal of the Chemical Society, Perkin Transactions I*, 1982, 2525.
6. Haddock, A., Gupta, R. K., Al-Shafi, S. M. and Haslam, E., *Journal of the Chemical Society, Perkin Transactions I*, 1982, 2515.
7. Nawwar, M. A. M., Souleman, A. M. A., Buddrus, J. and Linscheid, M., *Tetrahedron Letters*, 1984, **25**, 49.
8. Nawwar, M. A. M. and Hussein, S. A. M., *Phytochemistry*, 1994, **36**, 1035.
9. Nawwar, M. A. M., El-Mousallamy, A. M. D., Barakat, H. H., Buddrus, J. and Linscheid, M., *Phytochemistry*, 1989, **28**, 3201.
10. Harborne, J. B., *Phytochemical Methods*. Chapman and Hall, London, 1973, p. 45.
11. Nawwar, M. A. M., Souleman, A. M. A., Buddrus, J. and Linscheid, M., *Phytochemistry*, 1984, **23**, 2347.

12. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of the Flavonoids*. Springer, New York, 1969.
13. Nawwar, M. A. M., Hussein, S. A. M. and Merfort, I., *Phytochemistry*, 1994, **36**, 793.
14. Markham, K. R., Chari, V. M. and Mabry, T. J., *The Flavonoids, Advances in Research*, ed. J. B. Harborne and T. J. Mabry, Chapman and Hall, London, 1982.
15. Joseph, M. I., Pichon, N. and Raynand, J., *Pharmazie*, 1987, **42**(H.2), 142.
16. Okuda, T., Hatano, T., Ogawa, N., Kira, R. and Matsuda, M., *Chemical and Pharmaceutical Bulletin*, 1984, **32**(11), 4662.
17. Hatano, T., Kira, R., Yasuhara, T. and Okuda, T., *Chemical and Pharmaceutical Bulletin*, 1989, **37**(8), 2083.
18. Souleman, A. M. A., Barakat, H. H., El-Mousallamy, A. M. D., Marzouk, M. S. A. and Nawwar, M. A. M., *Phytochemistry*, 1991, **30**(11), 3763.
19. Averett, J. E., Kerr, B. J. and Raven, P. H., *American Journal of Botany*, 1978, **65**(5), 567.
20. Averett, J. E., Raven, P. H. and Becker, H., *American Journal of Botany*, 1979, **66**(10), 1151.