Pergamon

PII: S0031-9422(96)00888-6

Phytochemistry, Vol. 45, No. 4, pp. 819–823, 1997

0031-9422/97 $17.00+0.00

TANNINS FROM THE LEAVES OF *PUNICA GRANATUM*

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(*Received in revised form* 21 *October* 1996)

Key Word Index—*Punica granatum*; Punicaceae; pomegranate; 1,2,4-trigalloylglucose; 1,3,4-trigalloylglucose; 1,4-digalloylglucose 3,6-hexahydroxydiphenylglucose; brevifolin carboxylic acid monopotassium sulphate; tannins; NMR.

Abstract—Leaves of *Punica granatum* contain the new gallotannins, 1,2,4-tri-*O*-galloyl-β-glucopyranose and 1,3,4-tri-*O*-galloyl-β-glucopyranose together with the hitherto unknown ellagitannins, 1,4-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenyl-β-glucopyranose and brevifolin carboxylic acid 10-monopotassium sulphate. Structures were established by conventional methods of analysis and confirmed by ^{1}H, ^{13}C NMR, 2D-chemical shift correlation NMR and ESI-MS (negative mode) spectrometric analysis. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of chemical studies of the polyphenolics of *Punica granatum* L. in Egypt, we have reported the isolation and structural elucidation of 19 constituents, including flavone glycosides and gallo- and ellagitannins from leaf tissue [1, 2]. In the present communication we describe the isolation and structural elucidation of the new galloyl glucoses, 1,2,4-tri-*O*-galloyl-β-glucopyranose (**1**) and 1,3,4-tri-*O*-galloyl-β-glucopyranose (**2**) together with the new ellagitannins, 1,4-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenyl-β-glucopyranose (**3**) and brevifolin carboxylic acid 10-monopotassium sulphate (**4**).

The new compound **4** is of special interest as it represents the first reported natural occurrence of an ellagitannin bearing a potassium sulphate residue. However, sulphated derivatives are known in association with flavonoid chemistry and several flavonoid potassium sulphates are reported in the current literature [3].

RESULTS AND DISCUSSION

Compound **1**, was isolated as a white amorphous powder, which possessed galloyl ester-like characters (intense blue $FeCl_3$ reaction, rosy red colour with KIO_3 [4] and characteristic UV spectral maxima in methanol, Table 1). ESI-mass spectrometry analysis (negative mode) established that **1** was a tri-galloylglucose ($[M-H]^-$, *m/z* 635) with a M_r of 636. On normal acid hydrolysis (2 N aqueous HCl, 3 hr, 100°) **1** yielded gallic acid (CoPC, UV, ^{1}H and ^{13}C NMR spectral analysis) together with glucose (CoPC). The ^{1}H NMR spectrum of **1**, recorded in DMSO-d_6, at room temperature was assigned on the basis of the coupling constants, which were then confirmed by COSY NMR measurements. The proton resonances due to the glucose moiety were found at δ 5.98 (*d*, *J* = 8 Hz, anomeric H-1 in β-glucose moiety), 5.15 (*t*, *J* = 8 Hz, H-2), 5.0 (*t*, *J* = 8 Hz, H-4), 4.05 (*t*, *J* = 8 Hz, H-3), 3.87 (*m*, H-5) and at δ 3.45 (*m*, 2H-6) ppm. The resonances of the three galloyl moieties of **1** appeared as three singlets, each integrated to two protons at δ 7.0, 6.94 and 6.90 ppm. From these data it is evident that **1** is a trigalloylglucose, whose anomeric hydroxyl group is galloylated. Attachment of the remaining two galloyl moieties to the glucose hydroxyl groups at C-2 and C-4 unequivocally follows from the strong downfield shift of the corresponding geminal proton signals, a shift which is not observed for the resonances of H-3 and H-6 protons. Thus, the structure of **1** is confirmed as 1,2,4-tri-*O*-galloyl-β-glucose.

The ^{13}C NMR data of **1** are also in accordance with this structure. Thus, the β-anomeric carbon was recognized from the resonance at δ 92.9 ppm, being shifted upfield in comparison with the resonance of the corresponding carbon in the spectrum of unsubstituted β-glucose. This shift is due both to galloylation of the anomeric hydroxyl group and to galloylation of the vicinal hydroxyl at C-2 (β-effect). Galloylation at C-2 and C-4 follows from the upfield shifts of the resonances of C-1, C-3 and C-5, compared to

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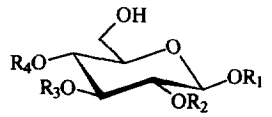
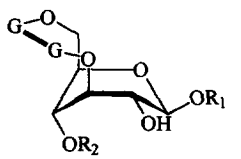
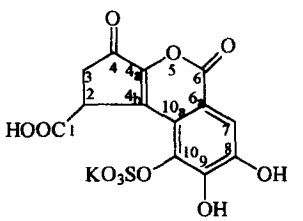


Compound 1 : $R_1 = R_2 = R_4$ = galloyl, R_3 = H
Compound 2 : $R_1 = R_3 = R_4$ = galloyl, R_2 = H



Compound 3 : $R_1 = R_2$ = galloyl
G—G = hexahydroxydiphenyl



Compound 4

the resonances of the corresponding carbons in free β-glucopyranose [5]. These β-effects have been observed, previously in similar cases [6]. The presence of three galloyl moieties in **1** follows from the three carbonyl carbon resonances, recorded in this spectrum at δ 165.0, 165.8 and 165.9 ppm. Other resonances of the remaining galloyl carbons were found to possess similar chemical shifts to those reported for other galloyl glucoses [6, 7]. Furthermore, the measured chemical shift values of the glucose carbon resonances, in the spectrum of **1**, confirmed that the sugar moiety is present in the pyranose form. Consequently, **1** is identified as 1,2,4-tri-*O*-galloyl-β-glucopyranose, which is a new natural product.

The second compound, **2**, was proved through chromatographic, UV spectral, hydrolytic data and ESI-mass spectrometry (negative mode) to be a positional isomer of **1**. Characterization of this minor constituent was completed by ^{1}H and COSY NMR analysis, as well as by comparing its NMR data with those reported previously for similar galloyl glucoses [1, 6, 7]. The ^{1}H NMR spectrum of **2** revealed three aromatic proton singlets, each integrated to two protons, at δ 6.98, 6.84 and 6.80 ppm, assignable to the three existing galloyl moieties in the molecule of **2**. The spectrum also showed in the sugar region three clearly resolved downfield proton resonances, the most downfield one of which was found to resonate at δ 5.79 ppm, attributable to a β-configurated anomeric glucose proton, due to its large coupling constant of 8.3 Hz. The remaining two downfield resonances were recognized at δ 5.39 and 5.12 ppm, each as a triplet, integrated to one proton, assignable to H-3 and H-4 glucose protons, respectively, and confirmed by the measurement of a COSY spectrum. The significant downfield shifts, recognized for these three resonances (in comparison with the resonances of the corresponding protons in free β-glucose [8]) indicated that the hydroxyl groups, geminal to their protons are galloylated. The spectra showed also the resonances of the H-2 proton at δ 3.77 ppm (*t*, J = 8.3 Hz) and those of H-5, H-6 and H-6′ protons as a multiplet at δ 3.36–3.58 ppm, thus proving the absence of galloylation at both the 2 and 6 hydroxyls. Compound **2** is therefore identified as 1,3,4-tri-*O*-galloyl-β-glucopyranose, which has not been reported previously as a natural product.

Compound **3** was isolated as a white amorphous powder of $[\alpha]_D$ −118° (ethanol; *c* 0.45) and showed the chromatographic properties, colour reactions

Table 1. Chromatographic and UV data of compounds **1**–**4**

Compound	Chromatographic properties R_f (× 100) H_2O	$HOAc–H_2O$	BAW	UV spectral data λ_{max}^{MeOH} (nm)
1	30	34	58	277
Gallic acid	53	59	78	272
2	32	36	56	277
3	26	30	28	275
$\mathbf{3}_a$	73	78	21	225, 260 shoulder
$\mathbf{3}_b$	55	59	32	276
Ellagic acid	00	09	46	255, 362
4	56	57	53	276, 346
Brevifolin carboxylic acid	55	59	62	278, 350, 362 shoulder

(dark blue $FeCl_3$ reaction and violet with nitrous acid test, specific for ellagitannins) and UV spectral data of an ellagitannin [8]. It exhibited a molecular ion peak at $[M-H]^-$, m/z 785, in negative ESI-MS, corresponding to a M_r of 687. On complete acid hydrolysis **3** yielded glucose, gallic and ellagic acids. The released ellagic acid, precipitated from the cold aqueous hydrolysate was characterized by UV, ^1H and ^{13}C NMR spectral analysis, while the identity of the released gallic acid, extracted from the hydrolysate by ether, was confirmed by CoPC, UV and ^1H NMR spectral analysis. On controlled hydrolysis **3** yielded, among other products, two major intermediates, $\mathbf{3}_a$ and $\mathbf{3}_b$. Both intermediates were purified by polyamide column fractionation using ethanol–water mixtures of decreasing polarities for elution. The identity of $\mathbf{3}_a$ as 3,6-(*R*)-hexahydroxydiphenyl-(α/β)-glucopyranose and that of $\mathbf{3}_b$ as 1-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenyl-β-glucopyranose, or corilagin [2] was confirmed by CoPC, UV, ^1H and ^{13}C NMR spectral analysis. This and the aforementioned data proved that **3** is a monogalloylated corilagin derivative. To determine the site of attachment of the galloyl moiety to corilagin in the molecule of **3** ^1H NMR analysis was employed. The spectrum revealed in the sugar region indicates that it is a tetra-*O*-substituted β-glucose [2]. Assignments of these resonances was made according to the mode of splitting, coupling constants and by comparison with the corresponding resonances in the spectrum of corilagin and its 2,4-digalloylated derivative, or punicafolin [2]. The β-anomeric proton resonances, appearing as a doublet was readily identified from its characteristic coupling constant, $J = 7.3$ Hz. However, the chemical shift value recorded for this doublet (δ 5.96 ppm) was found to be upfield with respect to that of the anomeric β-glucose proton in the spectrum of either corilagin or punicafolin (δ 6.20 and 6.38 ppm, respectively). This recognizable upfield shift reflected the absence of galloylation at the vicinal hydroxyl group, OH-2 and proved, therefore, galloylation at the only remaining alternative group OH-4. However, of more importance was its confirmation of the β-glucose conformation of **3**, which allows the β-anomeric proton to face the plane of the aromatic ring in the axially configurated galloyl moiety at C-4, thus shifting the anomeric proton resonance apparently upfield. Galloylation at OH-4 was further evidented by the lowfield location of its geminal proton signal to δ 4.77 ppm (*d*, $J = 3$ Hz, H-4). The resonances of the remaining glucose protons H-2, H-3, H-5, H-6 and H-6′, located in this spectrum at δ 4.07 (*m*), 5.53 (*d*, $J = 3$ Hz), 4.45 (*m*), 4.07 (*m*) and 4.45 (*m*), respectively, together with the recognized two galloyl proton singlets (each integrated to two protons) at δ 7.03 and 7.05 ppm and the two hexahydroxydiphenyl proton singlets (each integrated to one proton), were in full agreement with the achieved structure of **3** as 1,4-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenyl-β-glucose.

Final confirmation of this assigned structure was obtained by ^{13}C NMR analysis. As expected, the spectrum revealed two galloyl carbonyl carbon resonances at δ 164.56 and 164.70 ppm in addition to two hexahydroxydiphenyl carbonyl carbon resonances at δ 166.14 and 167.48 ppm. The β-anomeric glucose carbon was recognized from the aliphatic lowfield resonance at δ 93.53 ppm, while the most upfield resonance, at δ 63.89 ppm was assigned to the methylenic glucose carbon C-6. Assignments of the remaining glucose carbon resonances were aided by comparison with the previously recorded chemical shifts of corilagin and punicafolin [2]. In the spectrum of **3** the resonances of the remaining galloyl and hexahydroxydiphenyl carbons exhibited almost identical chemical shifts as those in corilagin and punicafolin, thus confirming the final structure of **3** as 1,4-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenyl-β-glucopyranose, a new ellagitannin which has not been reported previously in nature.

Compound **4** was isolated as yellow brown amorphous powder of no optical activity in methanol, and found to possess chromatographic properties, colour reactions (yellow spot under UV light on PC, dirty green colour with $FeCl_3$ and violet colour with nitrous acid spray reagent, Table 1) and UV spectral data which suggested that it was a brevifolin carboxylic acid derivative [2]. Controlled acid hydrolysis of **4** led to the release of brevifolin carboxylic acid only (CoPC, UV, ^1H and ^{13}C NMR data) and no other organic intermediates were detected. However, a heavy white precipitate was formed on treating the hydrolysate with aqueous $BaCl_2$, while on treating an aqueous solution of **4** with a solution of Na cobalt nitrite a yellow precipitate was formed. On electrophoretic analysis **4** migrated towards the anode, thus proving its anionic character. These data led to the suggestion that **4** exists as previfolin carboxylic acid monopotassium sulphate. The presence of potassium in the molecule of **4** was then confirmed by atomic absorption spectral analysis, while the M_r of 410 of **4** was confirmed by ESI-MS, which gave an anion at m/z 371, $[M-K]^-$, a base peak ion at m/z 291, $[M-KSO_3]^-$ and another ion at m/z 135, $[KSO_4]^-$. The latter ion is indicative of the potassium sulphate function [9].

To determine how the inorganic residue is incorporated in the molecule of **4**, both ^1H and ^{13}C NMR analyses were carried out. The ^1H NMR spectrum was similar to that of the parent compound, brevifolin carboxylic acid [2] and showed three aliphatic resonances at δ 4.37 (*dd*, $J = 2$ and 8 Hz) from the methinic proton H-2, 2.95 (*dd* $J = 8$ and 18 Hz) attributable to one of the methylenic protons, H-3 and 2.55 ppm, mostly hidden by the DMSO-d_6 signal, assignable to the other methylenic proton, H-3′. The aromatic, H-7 proton revealed itself, in this spectrum at δ 7.20 ppm. The close similarities of chemical shift values of the proton resonances of **4** to those of the corresponding resonances of the parent compound protons would suggest that the location of the potassium

sulphate residue in **4** is not interfering with these protons and, therefore, is not affecting their resonances.

^{13}C NMR spectral analysis finally solved the ambiguity of the site of attachment of the KSO_3 residue in **4** and afforded a spectrum in which 13 distinct resonances were recognized with chemical shift values similar to those recorded in the spectrum of brevifolin carboxylic acid. However, a distinction can be made since the resonances of C-9, C-10 and C-10a (see formulae) were found to possess different chemical shifts from those of the corresponding resonances in the spectrum of brevifolin carboxylic acid (upfield shift of C-10 to δ 142.92, downfield shifts of both C-9 to δ 142.74 and C-10a to δ 141.04). These changes in chemical shifts are obviously due to the attachment of the KSO_3 residue at C-10 of **4**. The absence of ^{13}C NMR resonances in the spectrum of **4**, apart from those of the brevifolin carboxylic acid moiety added further support for the presence of an inorganic KSO_3 residue. Thus, **4** is identified as brevifolin carboxylic acid 10-monopotassium sulphate. This is the first report of a naturally occurring sulphated ellagitannin.

That **1–4** are not artifacts was confirmed by 2DPC of the fresh crude leaf extract and subsequent routine analysis (CoPC against the identified compounds and measurement of UV spectra) of the purified spots, from multiple chromatograms, corresponding to each compound.

EXPERIMENTAL

^1H NMR spectra were measured at 400 MHz. ^1H resonances were measured relative to TMS and ^{13}C resonances to DMSO-d_6 and converted to the TMS scale by adding 39.5. Typical conditions: spectral width = 6000 Hz for ^1H and 22000 Hz for ^{13}C, 32K data points and a flip angle of 45°. ESI-MS (negative mode): the direct flow injection technique was applied, sample in MeOH was introduced (1.25 μl min^{-1}) together with MeOH sheath-liquid (5 μl min^{-1}) by a Harvard infusion pump 9 ml min^{-1} SF_6 sheath gas into the ESI ion source of a Finnigan MAT 4600 spectrometer. PC was carried out on Whatman no. 1 paper, using solvent systems: (1) H_2O; (2) HOAc–H_2O (3:47); (3) *n*-BuOH–HOAc–H_2O (4:1:5, upper layer); (4) C_6H_6–*n*-BuOH–H_2O–pyridine, 1:5:3:3, upper layer). Solvents 2 and 3 were used for prep. PC on Whatman 3 MM paper and solvents 3 and 4 for sugar analysis.

Plant material and fractionation. Leaves of *P. granatum* L. were collected from a mature tree, growing in Manfalout, upper Egypt, National Research Centre (NRC), Cairo, Egypt. A voucher specimen is deposited at the herbarium of the NRC. An aq. EtOH extract (3:1) of *P. granatum* L. leaves was worked up as described in ref. [2].

Isolation and identification. Pure **1–4** were isolated from the crude 90% EtOH fr. from a polyamide column by refractionation over Sephadex LH-20, using H_2O as eluent, followed by prep. PC of the eluents using solvent 3.

1,2,4-*Tri*-O-*galloyl*-β-*glucopyranose* (**1**). R_f values: Table 1. UV data: Table 1. M_r 636, ESI-MS (neg. ion): *m/z* 635 $[M-H]^-$. On complete acid hydrolysis (33 mg in 10 ml aq. 2 N HCl, 100°, 3 hr), **1** yielded glucose (CoPC) and gallic acid. The latter was extracted by EtOAc and purified through crystallisation from H_2O. Gallic acid: R_fs and UV data: Table 1; ^1H NMR: δ 6.98 (2H, *s*, H-2 and H-6); ^{13}C NMR: δ 120.6 (C-1), 108.8 (C-2 and C-6), 145.5 (C-3 and C-5), 138.1 (C-4), 167.7 (C=O). ^1H NMR of **1**: β-glucopyranose moiety: δ 5.98 (*d*, J = 8 Hz, H-1), 5.15 (*t*, J = 8 Hz, H-2), 5.0 (*t*, J = 8 Hz, H-4), 4.05 (*t*, J = 8 Hz, H-3), 3.87 (*m*, H-5), 3.45 (*m*, 2H-6); galloyl moieties: δ 7.0 (*s*), 6.94 (*s*), 6.90 (*s*). ^{13}C NMR of **1**: β-glucopyranose moiety: δ 92.9 (C-1), 72.2 (C-2), 73.4 (C-3), 71.5 (C-4), 76.5 (C-5), 61.1 (C-6); galloyl moieties: δ 121.4, 120.0, 119.8 (C-1), 109.5, 109.3, 109.2 (C-2 and C-6), 146.6, 146.5, 146.2 (C-3 & C-5), 140.3, 139.4, 139.3 (C-4), 165.9, 165.8, 165.0 (C=O).

1,3,4-*Tri*-O-*galloyl*-β-*glucopyranose* (**2**). R_fs: Table 1. UV data: Table 1. M_r 636, ESI-MS (neg. ion): *m/z* 635 $[M-H]^-$. On complete acid hydrolysis [16 mg in 5 ml aq. 2 N HCl, 100°, 3 hr], **2** yielded glucose and gallic acid (CoPC). ^1H NMR of **2**: β-glucopyranose moiety: δ 5.79 (*d*, J = 8.3 Hz, H-1), 3.77 (*t*, J = 8.3 Hz, H-2), 5.39 (*t*, J = 8.3 Hz, H-3), 5.12 (*t*, J = 8.3 Hz, H-4), 3.36-358 (*m*, H-5 and 2H-6); galloyl moieties: δ 6.98 (*s*), 6.84 (*s*), 6.80 (*s*).

1,4-*Di*-O-*galloyl*-3,6-(R)-*hexahydroxydiphenyl*-β-*glucopyranose* (**3**). R_fs: Table 1. UV data: Table 1. M_r 786, ESI-MS (neg. ion): *m/z* 785 $[M-H]^-$. $[\alpha]_D$ −118° (EtOH; c 045). On complete acid hydrolysis [64 mg in 15 ml aq. 2 N HCl, 100°, 3 hr], **3** yielded glucose, gallic acid (CoPC for both and UV data and ^1H NMR of the latter) and ellagic acid. Precipitated ellagic acid: R_fs: Table 1; UV data: Table 1; ^1H NMR: δ 7.5 (*s*); ^{13}C NMR: identical to those reported by us for ellagic acid in ref. [2]. On controlled hydrolysis (78 mg in 25 ml dist. H_2O, 100°, 3 hr), **3** yielded 3,6-(*R*)-hexahydroxydiphenyl-(α/β)-glucopyranose ($\mathbf{3}_a$) and corilagin ($\mathbf{3}_b$). Individual separation of $\mathbf{3}_a$ and $\mathbf{3}_b$ was achieved through CC of the onc. cold. aq. hydrolysate over polyamide, using aq. EtOH 20 and 30%, respectively) as solvent. $\mathbf{3}_a$: R_fs: Table 1. UV data: Table 1. ^1H NMR: α-glucopyranose moiety: δ 5.02 (*d*, J = 1.1 Hz, H-1), 3.98 (*br s*, H-2), 5.04 (*dd*, J = 4.4 and 2.2 Hz, H-3), 4.1 (*m*, H-4), 3.8 (*br s*, H-5), 4.1 (*m*, 2H-6); β-glucopyranose moiety: δ 5.32 (*d*, J = 4.22 Hz, H-1), 4.18 (*d*, J = 4.31 Hz, H-2), 5.2 (*t*, J = 4.8 Hz, H-3), 4.1 (*m*, H-4), 3.8 (*br s*, H-5), 4.1 (*m*, 2H-6); hexahydroxydiphenyl moiety: δ 6.62 (*s*), 6.60 (*s*), 6.54 (*s*), 6.48 (*s*). ^{13}C NMR: α-glucopyranose moiety: δ 102.1 (C-1), 79.6 (C-2), 77.3 (C-3), 76.5 (C-4), 70.0 (C-5), 65.7 (C-6); β-glucopyranose moiety: δ 95.5 (C-1), 79.5 (C-2), 77.1 (C-3), 73.5 (C-4), 70.0 (C-5), 65.5 (C-6); hexahydroxydiphenyl moiety: δ 123.0, 123.3, 123.8, 124.0 (C-1 and C-1′), 106.4, 106.7, 106.8, 107.2 (C-2 and C-2′), 144.0, 144.2, 144.6, 144.7 (C-3, C-3′, C-5

and C-5′), 135.1, 135.3, 135.4, 135.6 (C-4 and C-4′), 115.3, 115.38, 115.7, 115.8 (C-6 and C-6′), 166.2, 166.4, 168.1, 168.2 (C=O groups). **3**$_b$: R_fs: Table 1. UV data: Table 1. ^{1}H NMR: β-glucopyranose moiety: δ 6.20 (*d*, *J* = 7.3 Hz, H-1), 3.88 (*dd*, *J* = 7.3 and 3.2 Hz, H-2), 4.58 (*br s*, H-3), 4.22 (*br s*, H-4), 4.35 (*t*, *J* = 7.3 Hz, H-5), 4.25 (*dd*, *J* = 11.2 and 6.2 Hz, H-6), 3.95 (*dd*, *J* = 11.2 and 6.2 Hz, H-6′); galloyl moiety: δ 7.03 (*s*); hexahydroxydiphenyl moiety: δ 6.49 (*s*), 6.58 (*s*). ^{1}H NMR of **3**: β-glucopyranose moiety: δ 5.96 (*d*, *J* = 7.3 Hz, H-1), 4.07 (*m*, H-2), 5.53 (*d*, *J* = 3 Hz, H-3), 4.77 (*d*, *J* = 3 Hz, H-4), 4.45 (*m*, H-5), 4.07 (*m*, H-6), 3.45 (*m*, H-6′); galloyl moieties: δ 7.03 (*s*), 7.05 (*s*); hexahydroxydiphenyl moiety: δ 6.62 (*s*), 6.53 (*s*). ^{13}C NMR of **3**: β-glucopyranose moiety: δ 93.53 (C-1), 71.05 (C-2), 73.55 (C-3), 64.53 (C-4), 75.61 (C-5), 63.89 (C-6); galloyl moieties: δ 118.3, 118.6 (C-1), 109.3, 109.12 (C-2 and C-6), 145.60, 145.69 (C-3 and C-5), 139.20, 139.28 (C-4), 164.56, 164.70 (C=O groups); hexahydroxydiphenyl moiety: δ 122.99, 123.23 (C-1 and C-1′), 106.53, 107.13 (C-2 and C-2′), 143.97, 144.26, 144.95 (C-3, C-3′, C-5 and C-5′), 135.43, 135.90 (C-4 and C-4′), 115.14, 115.97 (C-6 and C-6′), 166.14, 167.48 (C=O groups).

Brevifolin carboxylic acid 10-monopotassium sulphate (**4**). R_fs: Table 1. UV data: Table 1. M_r 410, ESI-MS (neg. ion): *m/z* 371 $[M-K]^-$. Electrophoretic analysis: **4** was examined by electrophoresis on 3 MM Whatman paper at pH 2 using HOAc–HCO_2H buffer (H_2O–HOAc–HCO_2H, 89.5:8:2.5) at 50 V cm^{-1} for 90 min, mobility: 2.3 cm. On controlled acid hydrolysis [49 mg in 10 ml aq. 0.05 N HCl, 100°, 3 min], **4** yielded brevifolin carboxylic acid. The hydrolysate gave a white ppt. with aq. $BaCl_2$ assumed to be $BaSO_4$. Brevifolin carboxylic acid (extracted by EtOAc from the hydrolysate and crystallized from H_2O): R_fs: Table 1. UV data: Table 1. ^{1}H NMR: δ 4.35 (*t*, *J* = 7.5 Hz, H-2), 2.75 (*br s*, 2H-3), 7.22 (*s*, H-7). ^{13}C NMR: δ 173.40 (C-1), 41.70 (C-2), 37.40 (C-3), 194.30 (C-4), 149.0 (C-4a), 115.40 (C-4b), 160.70 (C-6), 112.80 (C-6a), 108.10 (C-7), 144.30 (C-8), 141.50 (C-9), 146.10 (C-10), 140.10 (C-10a). ^{1}H NMR of **4**: δ 4.37 (*dd*, *J* = 8 and 2 Hz, H-2), 2.95 (*dd*, *J* = 18 and 8 Hz, H-3), 2.55 (H-3′), 7.20 (*s*, H-7). ^{13}C NMR of **4**: δ 172.52 (C-1), 42.35 (C-2), 37.60 (C-3), 194.97 (C-4), 148.4 (C-4a), 115.59 (C-4b), 160.98 (C-6), 112.62 (C-6a), 108.05 (C-7), 143.20 (C-8), 142.74 (C-9), 142.92 (C-10), 141.04 (C-10a).

Acknowledgement—This work was executed through a project of Deutsche Forschungsgemeinschaft for which our deep gratitude is expressed.

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