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AMARIINIC ACID AND RELATED ELLAGITANNINS FROM *PHYLLANTHUS AMARUS*

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Key Word Index—*Phyllanthus amarus*; Euphorbiaceae; ellagitannins; amariinic acid.

Abstract—Chemical examination of the polar extractives of the aerial parts of *Phyllanthus amarus* led to the isolation of amariinic acid, a novel ellagitannin, together with 1-*O*-galloyl-2,4-dehydrohexahydroxydiphenoyl-glucopyranose, elaeocarpusin, repandusinic acid A and geraniinic acid B. On the basis of chemical and NMR data, including long-range correlation experiments, the structure of amariinic acid was established as the ring-opened oxidized cyclohexentrione moiety of DHHDP attached to O-4 of the glucose core.

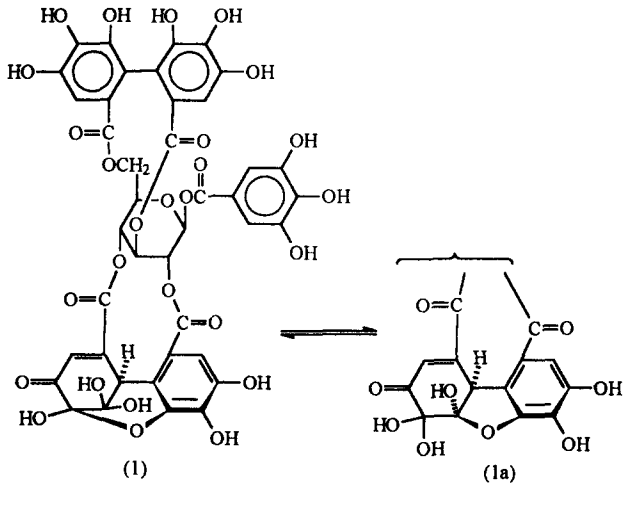
INTRODUCTION

Phyllanthus amarus is a small plant widely distributed in the tropics and highly valued by traditional practitioners for its healing properties [1–3]. Interest in this plant has been heightened by reports of antiviral activities and its potential as a remedy for hepatitis B viral infection [4–6]. In order to provide a chemical rationale for its popularity in traditional medicine, a detailed investigation of the polar, or aqueous, fraction of *P. amarus* was initiated. Such an investigation is desirable because the polar fraction was neglected in previous chemical studies [7–9] and, more importantly, this fraction provided the basis for many traditional uses and contained recently reported antiviral activities [6]. In earlier studies we reported that this fraction was dominated by ellagitannins with geraniin (**1**) as the principal plant metabolite [10]. Phyllanthusiin D (**2**) an unusual ellagitannin, was also isolated and characterized [11] but there is now some debate as to whether it is an artefact, as it can be produced by condensation between geraniin and acetone in weakly acidic medium [12]. Two novel dehydrohexahydroxydiphenyl (DHHDP) metabolites; namely amariin (**3**), which contains two DHHDP units bridged at O-2, O-4 and O-3, O-6 with the cyclohexentrione portion of the DHHDP units linked on to O-3 and O-6 of the sugar [10], and amarulone (**4**), an unusual cyclic compound postulated to be derived from amariin by oxidative cleavage of one of the hydrated cyclohexenetrione structures [13], were also found. In addition, the known 1,6-digalloylglucopyranose (**5**) and corilagin (**6**) were isolated and characterized. This report describes the isolation and structural elucidation of the remaining compounds in the aqueous extract.

RESULTS AND DISCUSSION

The 70% aqueous acetone extract of the aerial part of *P. amarus* was fractionated over a column of Sephadex LH20 using aqueous methanol to yield various fractions as previously reported [10]. Previously unresolved fractions were further subjected to repeated chromatographic treatment alternating between MCI gel CHP-20 using aqueous methanol and Sephadex LH20 with aqueous ethanol to afford compounds **7**–**12**. Compound **7** was finally purified in Sephadex LH20 using ethanol–water (3:17) as solvent. The ^{13}C NMR spectrum of **7** was unlike those encountered previously [10] but evidence of a galloyl moiety was apparent from the well-defined characteristic chemical shifts (δ110.4, 121.4, 139.3, 145.8) and the associated carbonyl carbon (δ167.9). In addition, two magnetically similar methylene carbons (δ39.4 and 40.0), three oxygenated methine carbons (δ66.5, 68.0 and 78.0) and an oxygenated quaternary carbon (δ76.7) were also observed in the spectrum together with an additional lower field carbonyl carbon (δ181.4) suggesting the presence of a quinic acid functionality. This was confirmed by the ^{1}H NMR spectrum and the carbon connectivity in the quinic acid structure was established by 2D NMR using ^{1}H–^{13}C and ^{1}H–^{1}H COSY. The latter method also showed that the low-field methine proton (δ4.92) attributed to the acylated position was coupled to the other two methine protons, establishing that the galloyl group was substituted on the hydroxyl at C-4 of the quinic structure. The 4-*O*-galloyl quinic structure was also confirmed by FAB-mass spectroscopy, which gave a peak at *m/z* 345 $[M + H]^+$, and by comparison of the NMR chemical shifts with published data [14].

Compound **8** was purified on MCI gel CHP 20P using 30% aqueous methanol as eluant. The ^{13}C NMR spectrum of **8** was highly complex with numerous peaks in the upfield region particularly in the region of the sugar carbon resonances. The presence of the sugar unit was established using ^{1}H–^{1}H COSY by making use of the diagnostic low-field position of the anomeric sugar proton chemical shift to reveal the position of the rest of the



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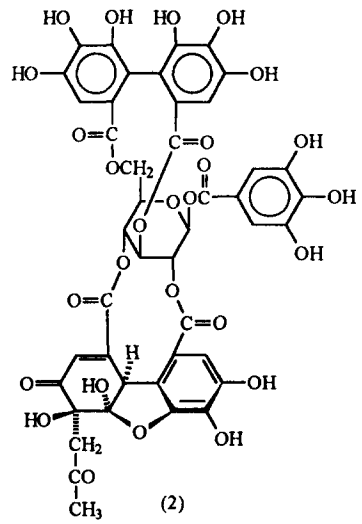

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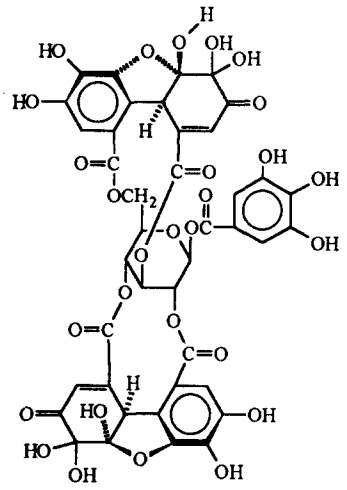

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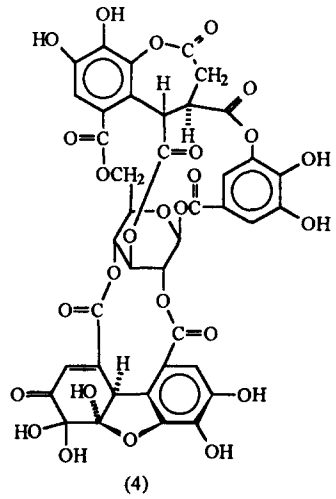
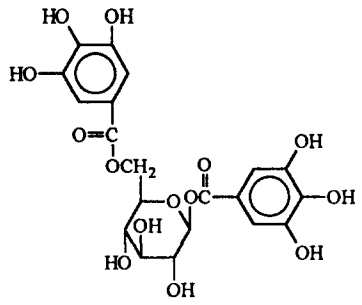


(4)

sugar protons. The presence of a galloyl unit was evidenced by the degenerate carbon and proton resonances in both the ^1H and ^{13}C NMR spectra. Evidence for a hexahydroxydiphenoyl moiety (HHDP) was also apparent from the pair of one proton singlets at δ6.92 and 7.06. The generally low-field position of the sugar proton signals indicated that all the hydroxyls were acylated and their small vicinal couplings showed that the sugar had the $^1C_4$ conformation brought upon by the HHDP moiety forming a bridge at O-2, O-4 or O-3, O-6. The latter positions were confirmed by mild hydrolysis with dilute acid which yielded corilagin (**6**). In addition, geraniin was also identified during the early stages of hydrolysis together with another highly mobile product (R_f 0.80 on cellulose TLC with 6% aqueous acetic acid) subsequently identified as ascorbic acid. Thus **8** was identified as elaeocarpusin, a natural component present in unusually high concentration in the leaves of *Elaeocarpus sylvestris* and shown to be a condensation product between ascorbic acid and the cyclohexentrione portion of the dehydrohexahydroxydiphenoyl group of geraniin [15, 16]. The structure of **8** was also confirmed by comparison of its ^{13}C NMR data with published results [15, 16].

Compounds **9** and **10** eluted out in the same fraction on MCl gel with 30% aqueous methanol as eluant. The fraction was concentrated and left to stand at ambient temperatures overnight, giving a mixture of yellow and colourless crystals. Fractional crystallization by redissolving in a small volume of methanol in H_2O with warming and then slow cooling, gave **9** as yellow crystals. The ^{13}C NMR spectrum of **9** showed multiplicity or pairing of carbon resonances, the presence of twin peaks (δ45.7 and 51.7) for the methine carbon, the accompanying pair of hemiacetal carbon chemical shifts (δ92.1 and 96.0) and the two low-field ketone carbonyl carbon signals (δ192.5 and 195.4) were indicative of the two equilibrating 5- and 6-membered ring structures of the DHHDP unit [17]. The complication associated with this multiplicity of NMR signals was overcome by the facile reaction of **9** with *o*-phenylenediamine which degraded the hemiacetal



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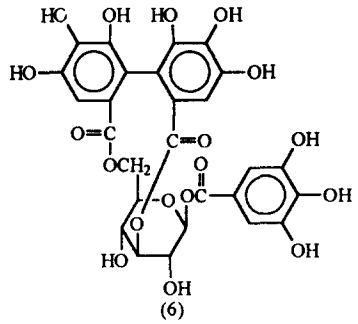

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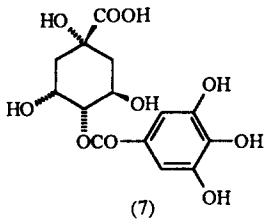

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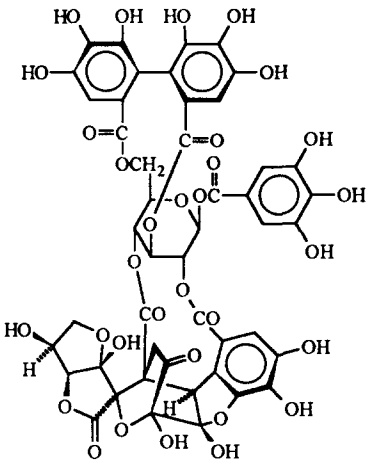

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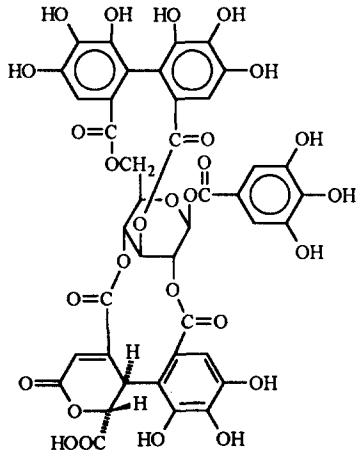

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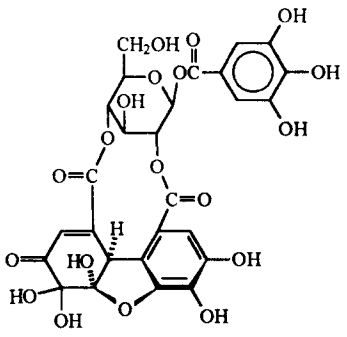
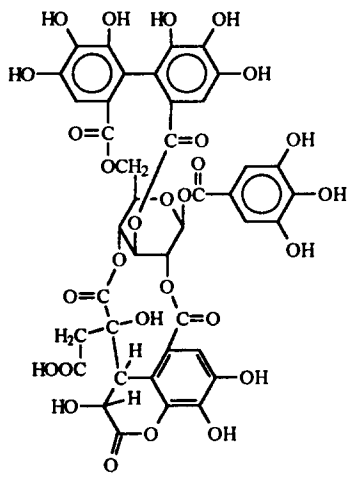


(9)

rings to form the corresponding phenazine derivative **13**. The NMR spectra of the phenazine (**13**) showed six well-resolved sugar carbon resonances and observation of two degenerate galloyl carbons together with the characteristic aromatic carbons of the phenazine structure were fully in accord with a hydrolysable tannin constitution for the parent compound consisting of galloyl and DHHDP substituents on a glucose core [10]. The location of these substituents on the sugar carbons was established using inverse long-range $^{1}H-^{13}C$ COSY optimized at $J = 8$ Hz (see Table 1). The anomeric proton (δ6.18) was observed to be long-range coupled to the low-field carbonyl carbon



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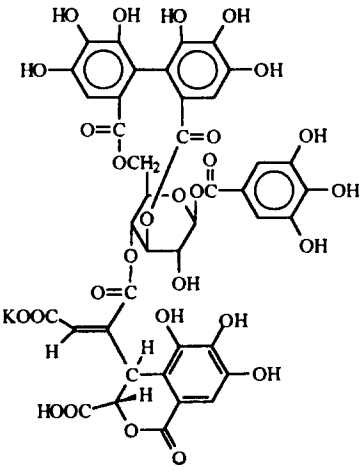

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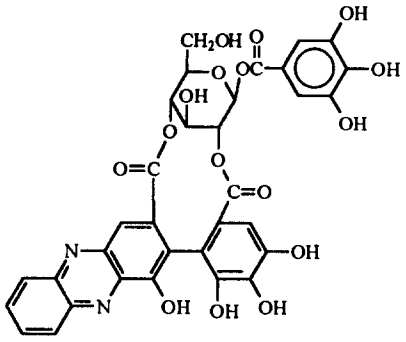

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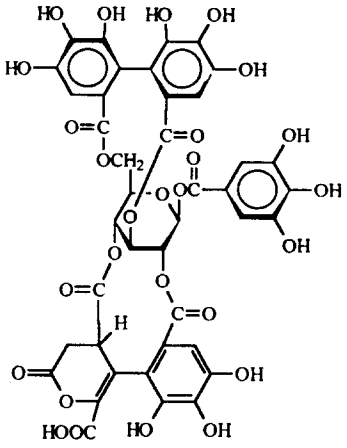

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Table 1. Inverse long-range HMBC of compound **13** (δin ppm)

H	Correlated carbons
4.01	73.4
4.52	62.7, 66.8, 73.4, 79.4, 80.5, 92.7
5.19	62.7 and 79.4
5.43	66.8, 73.4, 79.4, 168.2
6.18	66.8, 79.4, 165.0
6.99	110.0, 120.1, 139.6, 146.0, 165.0
7.52	116.4, 139.0, 145.3, 168.2
8.21	116.8, 135.9, 152.1, 167.2

(δ165.0) which in turn was associated with the degenerate galloyl protons (δ6.99), indicating that the galloyl substituent was at O-1 of the glucose core. The sugar H-2 (δ5.43) was similarly shown to be related to the carbonyl (δ168.2) attached to the galloyl substructure of the phenylphenazine moiety by virtue of its interaction with the aryl proton (δ7.52). The remaining carbonyl carbon (δ167.2) could not be assigned due to lack of interaction with any of the sugar protons, probably due to inappropriate selection of the J value for optimization. However, its location on the sugar could be established by examination of the sugar proton chemical shifts. The markedly downfield position of H-4 (δ5.19) with respect to H-3, H-5 and H-6 indicated the phenazine moiety must be attached at O-4. Thus, the DHHDP in the parent compound, **9** at O-2, O-4 was consistent with the small vicinal proton–proton couplings observed in the glucose core as a result of $^1C_4$ conformation. The parent compound, **9**, was therefore 1-galloyl-2,4-dehydrohexahydroxydiphenoyl-glucopyranose which was corroborated by FAB-mass spectroscopy giving a peak at m/z 649 [M − H]. Compound **9** is therefore identical to furosin, a component of *Geranium thunbergii* [17]. The 6-membered ring, hemiacetal structure of **9** in the crystalline material was confirmed by NMR using freshly prepared material in deuterated acetone as solvent. Under these conditions the more dominant ^{13}C NMR signals were the higher field carbons which were diagnostic of the 6-member ring methine (δ45.7) and carbonyl (δ192.5) carbons. Addition

of a drop of D_2O to the NMR sample changed the relative peak intensity of the two forms from a ratio of about 3:1 to 1:1, consistent with published results [17].

The ellagitannin constitution of **10** was apparent from its ^{13}C NMR spectrum with the observation of six sugar carbon signals in the region δ64.0–91.3 and characteristic galloyl and HHDP carbon resonances in the aromatic region. The small vicinal couplings observed for the sugar protons indicated that the pyranose ring assumed the quasi-boat or $^1C_4$ conformation and therefore the HHDP group must be bridged at either the O-2, O-4 or O-3, O-6 positions. In addition, an aliphatic methine carbon (δ37.8) and an oxygenated methine carbon (δ80.5) were also present in the ^{13}C NMR spectrum of **10** and the protons associated with these carbons appeared as broad singlets. However, ^1H–^1H COSY data indicated that they were coupled to each other. Evidence of direct bonding between these two methine carbons was also available from long range ^1H–^{13}C coupling experiments using HMBC (Table 2) which showed the proton (δ5.11) associated with the oxygenated carbon (δ80.5) correlated to the upfield methine carbon (δ 37.8). In addition, this proton was also associated with a HHDP substructure as evidenced by its long range ^1H–^{13}C couplings to the quaternary carbons (δ116.3 and 145.5) and the two carbonyl carbons (δ162.8 and 172.0) which were not directly linked to the sugar oxygens. The olefinic proton (δ6.44) was also observed to be long-range coupled to the upfield methine carbon (δ37.8) and to the carbonyl carbon (δ162.8) as well as another ester carbonyl carbon (δ164.9) associated with H-4 of the sugar. These data were consistent with a cyclic lactone structure linked to the sugar O-4 as shown in **10**. The small vicinal couplings between the two methine protons indicated a *trans* relationship with respect to their relative orientation. The structure **10** was also corroborated by FAB-mass spectroscopy (*m/z* 953 $[M + H]^+$) and by mild acid hydrolysis to yield corilagin (**6**). Compound **10** was therefore identical to geraniinic acid B which was reported to be a constituent of *Phyllanthus flexuosus* [12].

The novel compound amariinic acid, **11**, was purified on MCl gel column using 30% methanol as eluant. The ^{13}C NMR spectrum of **11** showed the characteristic glucose resonances with the anomeric carbon at δ91.9 and the remaining five carbon resonances in the higher field region (δ62 to 74). In addition, a galloyl group characterized by the two degenerate unsubstituted aromatic carbons (δ110.6) and a HHDP unit indicated by the two upfield unsubstituted aromatic carbons resonances (δ107.8 and 110.5) were also present. The small values of the vicinal proton couplings observed between the sugar protons showed the $^1C_4$ conformation for the sugar which was necessitated by the bridging of the HHDP unit at with O-2, O-4 or O-3, O-6. An additional lower field aromatic methine carbon resonance (δ116.3) was also present, suggesting an additional HHDP moiety in which only one aromatic ring remained intact. Evidence for the oxidative cleavage of the other ring of this HHDP unit was the observation of an additional oxygenated methine carbon (δ66.0), a quaternary oxygenated carbon (δ76.2), an upfield methine carbon (δ49.4) and a methylene carbon (δ41.7) together with seven carbonyl carbons, five of which could be accounted for as ester linkages to the sugar hydroxyls. Inverse long-range ^1H–^{13}C correlation experiments (HMBC) showed interaction between the sugar anomeric proton and the highest field carbonyl carbon (δ165.3), which in turn correlated to the degenerate galloyl protons (δ7.19), thus establishing the position of the galloyl moiety on the glucose (see Table 3). The HHDP protons (δ6.63 and 7.07) showed similar long-range correlations with the two carbonyl carbons (δ168.7 and 166.4 respectively) related to the sugar H-6 (δ4.41) and H-3 (δ5.94), respectively, indicating that the HHDP moiety was bridged at O-3 and O-6 on the sugar. These results were also confirmed by partial acid hydrolysis of **11** to yield corilagin (**6**) as the main product and gallic acid and glucose. The two methylene protons (δ2.83 and 3.40) in the 2D HMBC plot were observed to be associated with the quaternary oxygenated carbon (δ76.2) as well as the two low-field carbonyl carbons (δ172.1 and 172.7) indicating the bonding sequence O=C–CH_2–C–C=O. Furthermore, the oxygenated quaternary carbon (δ76.2) was long-range coupled to the methine carbon (δ66.0) whose proton was associated with two carbonyl carbons(δ166.6 and 172.7), the latter being

Table 2. Inverse long-range HMBC of compound **10** (δin ppm)

H(δ)	Correlated carbons
4.39	64.0
4.73	64.0, 66.0, 73.2, 91.3, 168.9
5.11	37.8, 116.3, 144.9, 145.5, 162.8, 172.0, 66.0 (W)
5.35	80.5, 116.3, 122.6, 144.8, 148.5, 172.0
5.36	66.0, 71.7, 73.2, 91.3
5.39	64.5, 66.0, 91.3, 166.2
5.50	64.5, 71.7, 166.5
6.44	37.8, 107.9, 116.3, 144.7, 162.8, 164.9
6.59	64.5, 71.8, 73.2, 165.6
6.70	115.2, 136.3, 145.5, 168.9
7.01	116.3, 138.6, 145.0, 166.5, 148.5,
7.08	117.1, 137.7, 166.5, 144.8
7.23	110.8, 119.8, 140.2, 145.9, 165.6

Table 3. Inverse long-range HMBC of compound **11** (δ in ppm)

H	Correlated carbons
2.83, 3.40	76.2, 172.1, 172.7
4.41	67.5, 73.9, 168.7
4.78	64.1, 67.5, 73.9, 91.9, 168.7
4.86	66.0, 76.2, 114.9, 119.8, 143.2, 166.6, 172.7
5.22	62.3, 71.1, 172.7
5.55	62.3, 67.5, 91.9, 166.2
5.94	67.5, 71.1, 73.9, 91.9, 166.4
6.53	62.3, 71.1, 73.9, 165.3
6.63	115.2, 136.4, 145.3, 168.7
7.07	117.1, 137.8, 144.8, 166.4, 124.4
7.19	110.6, 120.1, 140.0, 146.1, 165.3
7.54	114.9, 119.8, 139.4, 166.2, 146.1

coupled to the sugar H-4. This carbon was also related to several quaternary carbons (δ114.9, 119.8 and 143.2) which were identifiable with the intact half of the HHDP structure and hence very much like a chebulagic acid unit [18, 19]. This HHDP moiety was linked via an ester linkage to O-2 of the sugar, as shown by the long-range interactions between the sugar H-2 (δ5.55), the carbonyl carbon (δ166.2) and the methine proton (δ7.54) of the HHDP substructure. Compound **11** was therefore determined to be amariinic acid, which was also confirmed by FAB-mass spectroscopy (*m/z* 971 $[M + H]^+$). The configuration of the chiral centres of the alicyclic substructure could not be established from the proton coupling pattern due to coincidence of the proton chemical shifts of the two methine protons (δ4.86) attached to the two chiral carbons at δ66.0 and 49.4, but biosynthetic consideration would suggest a *cis* relationship as in the chebulagic acid unit [18, 19].

Compound **12** was highly mobile on both MCl gel and Sephadex LH 20 eluting out almost at void volume with dilute aqueous alcoholic solvents. The compound was highly soluble in water but not readily soluble in ethanol or acetone. It was finally purified on MCl gel using water as eluant. The ^{13}C NMR spectrum of **12** was very similar to that of geraniinic acid B with two oxygenated aliphatic carbon signals in addition to the sugar resonances, and an upfield methine carbon (δ35.78). The assignment of the H-2 sugar proton was readily made from ^1H–^1H COSY by its proton coupling to the anomeric proton and its relative upfield position (δ4.15) indicated that the attached hydroxyl was not acylated. The observation of a low-field methine proton at δ6.82 associated with a vinylic carbon (δ137.2), together with the presence of two methine carbons (δ35.8 and 81.0) and a carboxylic acid carbonyl (δ175.0), indicated the presence of a chebulic acid moiety. This was further corroborated by long-range ^1H–^{15}C COSY experiments (see Table 4) which also established a galloyl unit at O-1 and an HHDP function bridging at O-3 and O-6 on the glucose ring, indicating **12** to have a repandusinic acid A constitution. FAB-mass spectrometry *m/z* 1009 $[M + H]^+$ corresponding to the potassium salt of repandusinic acid A and identical to that isolated from *Mallotus repandus* [20]. The constitution of **12** was confirmed by comparison of ^{13}C NMR data with that of the potassium salt of repandusinic acid A [20]. The compound was found to be rather unstable in acid, as attempts to convert the salt to the acid form by addition of dilute HCl led to rapid degradation of **12** to corilagin.

Table 4. Inverse long-range HMBC of compound **12** (δ in ppm)

H	Correlated carbons
4.15	68.8
4.18	64.2
4.53	64.2, 73.5, 169.2
4.70	70.5
4.79	81.0, 35.8, 116.3, 135.2, 167.4, 175.0
5.32	35.8, 81.0, 116.3, 117.3, 135.2, 136.5, 143.9, 166.5, 175.0
5.38	64.2, 166.5
6.11	94.8, 73.5, 166.3
6.59	108.4, 115.5, 125.1, 136.5, 145.7, 169.2
6.69	109.2, 116.3, 137.2, 144.6, 167.4
6.82	137.2, 109.2, 116.3, 135.2, 166.5, 173.6
6.99	109.2, 116.3, 144.8, 167.4
7.00	110.8, 120.0, 139.4, 145.7, 166.3

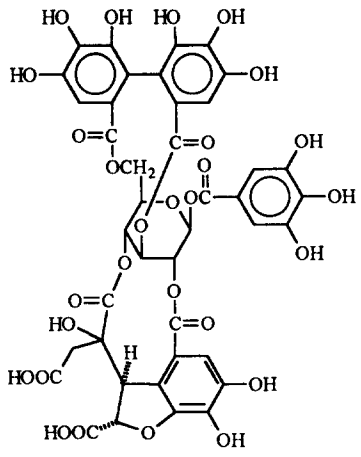
This chemical investigation of *Phyllanthus amarus* has shown geraniin to be the most abundant metabolite in the polar extracts, followed to a lesser extent by amariin, a closely related metabolite with the rather uncommon two DHHPD units linked to the same sugar core. The remaining hydrolysable tannin constituents, almost all of which were based on the $^1C_4$ glucose conformation due to bridging at O-2, O-4 or O-3, O-6 by HHDP or DHHDP groups, were present at much lower concentrations. These minor products could be viewed biosynthetically as arising from oxidative modification of the reactive DHHDP group to give rise to structures that could react further via esterification to give rise to diverse group of closely related compounds. Compounds such as amariinic acid, geraniinic acid B and repandusinic acid A are structurally related to one another and also to phyllanthusiin A (**14**), B (**15**), and C (**16**) from *Phyllanthus flexuosus* [12] as well as to mallotinin (**17**), a bicyclic lactone form *Mallotus repandus* [20]. In many respects the hydrolysable tannins profile of *Phyllanthus amarus*, conspicuous by the predominance of geraniin and complete absence of oligomeric analogues, is similar to that of *Geranium thunbergii* Sieb. et Zucc., a highly valued Japanese herb [17].

EXPERIMENTAL

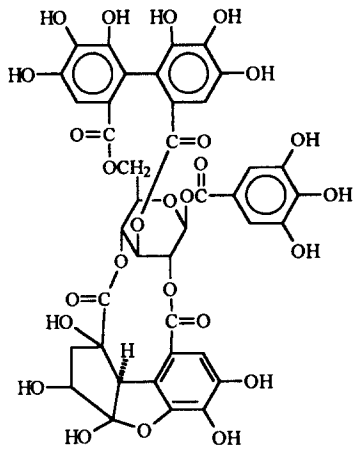
Hot house plants raised from seeds obtained from Madras, India were dried at ambient temp and the aerial portion of the plant extracted with Me_2CO–H_2O (7:3). The combined extracts were concd to give a dark coloured sludge, diluted with H_2O and filtered over glass wool. The filtrate was extracted with CH_2Cl_2 and the aq. layer concd to half volume and the residue applied directly to a Sephadex LH20 column prepd in H_2O. The column was eluted with H_2O and then with increasing proportions of MeOH in H_2O. Frs monitored by cellulose TLC plates developed with HOAc–H_2O (3:47) and visualized with ferric chloride–potassium ferricyanide spray. Frs showing similar spot pattern on cellulose TLC were combined and concd on a rotatory evaporator and the residue freeze-dried. Mixtures were further sepd by CC alternating between MCl gel (aq. MeOH) and Sephadex LH20 (aq. EtOH).

4-O-Galloylquinic acid (**7**). R_f 0.75 $[\alpha]_D$ $-13.7°$ (MeOH; *c* 0.02); FAB-MS *m/z*: 345 $[M + H]^+$. ^{13}C NMR (Me_2Co-d_6): δ39.4, 40.0, 66.5, 68.0, 76.7, 78.0, 110.4, 121.4, 139.3, 145.8, 167.9 and 181.4. ^1H NMR (Me_2CO-d_6): δ1.9–2.1 (*m*, H-2, H-6), 4.12 (*m*, H-3), 4.23 (*m*, H-5), 4.92 (*dd*, J = 7.3 and 12.8 Hz) and 7.10 (*s*, ArH).

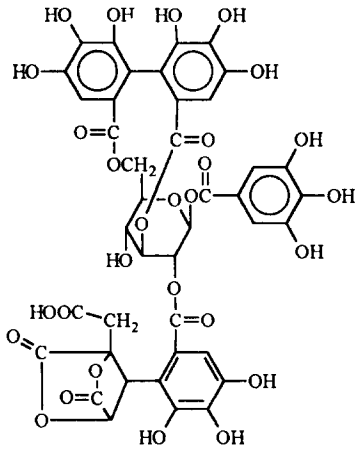
Elaeocarpusin (**8**). Freeze-dried to give an amorphous solid (42 mg) R_f 0.10, $[\alpha]_D$ $+47°$ (MeOH; *c* 0.01).



(15)



(16)



(17)

^{13}C NMR Me_2CO-d_6): δ37.9, 49.7, 51.8, 63.3, 64.3, 68.7, 73.5, 74.3, 76.4, 80.6, 89.4, 92.3, 96.3, 107.9, 109.0, 109.3, 109.8, 109.9, 110.1, 110.2, 110.4, 114.0, 116.3, 116.4, 116.9, 118.4, 120.2, 123.1, 124.9, 136.2, 137.2, 137.4, 139.7, 144.8, 144.9, 145.1, 145.2, 145.9, 146.0, 147.5, 148.6, 164.8, 165.3, 166.5, 168.1, 168.2, 170.7 and 197.9. ^{1}H NMR (Me_2CO-d_6): δ2.18 (*d*, J = 19 Hz), 3.02 (*d*, J = 19 Hz), 3.9–4.3 (*m*), 4.59–4.90 (*m*), 4.92 (*br s*, sugar H-3), 5.43 (*br s*, sugar H-2), 5.74 (*d*, J = 2.1 Hz), 5.97 (*br s*, sugar H-4), 6.53 (*d*, J = 3.6 Hz, sugar H-1), 6.92 (*s*), 7.06 (*s*), 7.20 (*s*, galloyl) and 7.26 (s).

Treatment of **8** in H_2O on a heated water bath for 30 mins and examination by 2D cellulose TLC confirmed the presence of ascorbic acid and geraniin.

Furosin (**9**). Yellow crystals from aq. MeOH, R_f 0.32 (HOAc–H_2O, 3:47) and 0.32 (t-BuOH–HOAc–H_2O, 3:1:1), $[\alpha]_D$ − 148° (MeOH; *c* 0.08). Negative ion FAB-MS *m/z*: 649 $[M - H]^-$. ^{13}C NMR (Me_2CO-d_6): δ45.7, 51.7, 62.2, 62.4, 62.5, 62.6, 70.9, 71.9, 72.0, 73.3, 77.6, 78.2, 91.7, 92.1, 92.6, 96.0, 108.7, 110.4, 113.5, 114.0, 115.8, 117.2, 118.7, 119.7, 119.9, 120.1, 125.7, 128.8, 137.2, 139.1, 139.9, 143.0, 145.5, 145.9, 146.9, 147.4, 149.1, 154.5, 165.5, 165.9, 166.1, 166.2 166.5, 192.5, and 195.4. ^{1}H NMR (Me_2CO-d_6): δ3.9–4.3 (*m*, sugar H-6), 4.35 (*m*, sugar H-5), 4.4–4.60 (*br s*, H-3), 4.94 (*br s*), 5.09 (*br s*, sugar H-4'), 5.17 (*br, s* sugar H-4'), 5.30 (*s*), 5.33 (*m*, sugar H-2), 6.30 (*br s*), 6.4–6.46 (*br s*, sugar H-1), 6.58 (*s*), 7.19, 7.21, 7.23 and 7.26.

The phenazine (**13**) was prepd as follows: **9** (20 mg) was added to a soln of *o*-phenylenediamine (10 mg) in 20% HOAc in EtOH (10 ml), shaken to dissolve and left overnight at ambient temp. Water (10 ml) was added and the resulting suspension concd on a rotatory evaporator. To the residue was added more H_2O and the orange phenazine (**13**) collected by filtration and dried at ambient temp. under vacuum, $[\alpha]_D$ − 251° (Me_2CO; *c* 0.3). ^{13}C NMR (Me_2CO-d_6): δ62.7, 66.8, 73.4, 79.4, 80.5, 92.7, 110.0, 110.3, 112.6, 116.4, 116.8, 119.4, 120.1, 121.3, 129.9, 130.2, 132.3 × 2, 135.9, 139.0, 139.6, 142.6, 142.8, 144.9, 145.3, 145.8, 146.0, 152.1, 165.0, 167.2 and 168.2. ^{1}H NMR (Me_2CO-d_6): δ4.01 (*m*, H-6, H-6'), 4.52 (*m*, H-3, H-5), 5.19

(*d*, J = 2.8 Hz, H-4), 5.43 (*d*, J = 5.7 Hz, H-2), 6.18 (*d*, J = 5.7 Hz, H-1), 6.99 (*s*, galloyl 7.52 (*s*), 7.88 (*br s*), 8.05 (*br s*) and 8.21 (*s*).

Geraniinic acid B (**10**). Crystalline material from aq. MeOH R_f 0.35 (HOAc–H_2O, 3.47 and 0.40 (*t*-(BuOH–HOAc–H_2O, 3:1:1), FAB-MS *m/z*: 953 $[M - H]^+$. ^{13}C NMR (Me_2CO-d_6): δ37.8, 64.0, 64.5, 66.0, 71.7, 73.2, 80.5, 91.3, 107.9, 110.0, 110.4, 110.8, 112.0, 115.2, 116.3, 117.1, 119.8, 122.6, 122.7, 124.2, 125.3, 136.3, 137.7, 138.6, 140.2, 144.7, 144.8, 144.9, 145.0, 145.5, 145.9, 146.0, 148.5, 162.8, 164.9, 165.5, 166.2 × 2, 168.9 and 172.0. ^{1}H NMR (Me_2CO-d_6): δ4.39 (*m*, H-6′), 4.73 (*m*, H-5, H-6), 5.11 (*br s*, methine H), 5.35 (*br s* methine H), 5.39 (*br s*, H-3), 5.50 (*m*, H-4), 6.44 (*s*, HHDP), 6.59 (*br s*, H-1), 6.70 (*s*, HHDP), 7.01 (*s*), 7.08 (*s*) and 7.23 (*s*, galloyl).

Amariinic acid (**11**). Amorphous freeze dried powder, R_f 0.23, $[\alpha]_D$ − 46° (MeOH; *c* 0.09); FAB-MS *m/z*: 971 $[M + H]^+$. ^{13}C NMR (Me_2CO-d_6): δ41.7, 49.4, 62.3, 64.1, 66.0, 67.5, 71.1, 73.9, 76.2, 91.9, 107.8, 110.5, 110.6, 114.9, 115.2, 116.3, 117.1, 119.8, 120.1, 124.4, 125.6, 136.4, 137.8, 139.4, 140.0, 143.2, 144.8, 145.0, 145.3, 145.5, 146.1, 146.2, 165.3, 166.2, 166.4, 166.6, 168.7, 172.1 and 172.7. ^{1}H NMR (Me_2CO-d_6): δ2.83 (*d*, J = 16.3 Hz methylene H), 3.40 (*d*, J = 16.3 Hz, methylene H), 4.41 (*m*, H-6), 4.78 (*m*, H-6′), 4.86 (*m*, 2 × methine H), 5.22 (*d*, J = 3.5 Hz. H-4), 5.55 (*br s*, H-2), 5.94 (*br s*, H-3), 6.53 (*br s*, H-1), 6.63 (*s*, HHDP) 7.07 (*s*, HHDP), 7.19 (*s*, galloyl) and 7.54 (HHDP).

Repandusinic acid B. Potassium salt (**12**) (82 mg), freeze-dried amorphous off-white R_f 0.27, $[\alpha]_D$ + 31° (MeOH; *c* 0.07); Positive ion FAB-MS *m/z*: 1009 $[M + H]^+$. ^{13}C NMR (Me_2CO-d_6): δ35.8, 64.2, 64.4, 68.8, 70.5, 73.5, 81.0, 94.8, 108.4, 109.2, 110.3, 110.8, 115.5, 116.3, 116.4, 117.3, 120.0, 124.8, 125.1, 135.2, 136.5, 137.2, 139.4, 139.9, 143.9, 144.6, 144.8, 144.9, 145.2, 145.7 166.3, 166.5, 167.4, 167.8, 169.2, 173.6, and 175.0. ^{1}H NMR (Me_2CO-d_6): δ4.15 (*br s*, sugar H-2), 4.18 (*m*, H-6), 4.53 (*m*, H-5, H-6′), 4.70 (*br s*, H-3), 4.79 (*d*, J = 1.0 Hz, methine H), 5.32 (*d*, J = 1.0 Hz, methine H), 5.38 (*m*, H-4), 6.11 (*d*, J = 3.1 Hz, H-1), 6.59 (*s*, HHDP), 6.69 (*s*, HHDP), 6.82 (*s*, vinyl H), 6.99 (*s*, HHDP) and 7.00 (*s*, galloyl H). Treatment of **12** with 0.1 N HCl at room temp for 2 hr gave corilagin as principal hydrolysis product.

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