Tannins and Related Compounds. Part 3.[1] A New Phenolic Acid, Sanguisorbic Acid Dilactone, and Three New Ellagitannins, Sanguiins H-1, H-2, and H-3, from *Sanguisorba officinalis*

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A new phenolic acid, sanguisorbic acid dilactone, and three new ellagitannins, sanguiins H-1, H-2, and H-3, have been isolated from the underground parts of *Sanguisorba officinalis* L. (Rosaceae). On the basis of chemical and spectroscopic evidence, sanguisorbic acid dilactone has been shown to possess a trimeric gallic acid structure and to be a structural isomer of valoneaic acid dilactone. Similarly, sanguiins H-1 and H-2 have been characterized as 1,6-digalloyl-2,3-(4,4',5,5',6,6'-hexahydroxydiphenoyl)glucose and 1-galloyl-2,4-(4,4',5,5',6,6'-hexahydroxydiphenoyl)-3,6-sanguisorboylglucose, respectively. Sanguiin H-3 has been shown mainly by spectroscopic data and analyses of partial hydrolysates to have a novel dimeric structure containing 2,3-hexahydroxydiphenoyl)-glucose and sanguiin H-2 moieties.

PROGRESS in elucidating the chemistry of the ellagitannins has been hampered by difficulties in their separation, purification, and structural identification. However, remarkable advances were made in the period 1950—1970, particularly by Schmidt and his collaborators who proposed structures for chebulagic acid,[1] chebulinic acid,[2] and terchebin[3] from myrobalans (*Terminalia chebula*) and brevilagins 1[4] and 2[5] from algarobilla (*Caesalpinia brevifolia*). We have recently isolated gallotannins from extracts of *Paeonia albifolia* Pallas var. *trichocarpa* Bunge,[6] ellagitannins from commercial cloves[7] and seed shells of *Trapa japonica* Flerove,[8] and proanthocyanidins from commercial rhubarbs[9] and the seeds of *Areca catechu* L.[10] by the use of Sephadex LH-20 dextran gel; the structures of these tannins were deduced by application of ^{1}H and ^{13}C n.m.r. spectroscopy. Similar techniques of isolation and analysis have now been extended to other tannins occurring in *Sanguisorba officinalis* L.; this has been known for some time to be a rich source of tannins of uncertain nature.

RESULTS AND DISCUSSION

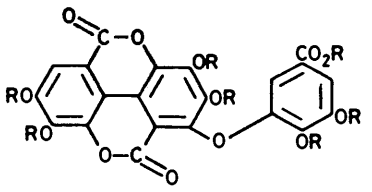
A new phenolic acid, sanguisorbic acid dilactone (1a), and three new ellagitannins, sanguiins H-1 (2), H-2 (3a), and H-3 (4a) were isolated from the aqueous acetone extracts of the fresh underground parts of *Sanguisorba officinalis* L. by a combination of adsorption (Sephadex LH-20) and partition (cellulose) chromatography. In addition, (+)-catechin, (±)-gallocatechin, procyanidins B-3 and C-2,[11] gallic acid, ellagic acid, and eugeniin[7] were obtained, and these were identified by comparisons with authentic samples.

Sanguisorbic acid dilactone (1a), m.p. >300 °C, $C_{21}H_{10}O_{13}$, $3H_2O$, is sparingly soluble in most organic solvent except for pyridine, and in this characteristic closely resembles ellagic acid. The presence of three aromatic rings (eighteen aromatic carbon signals), along with three aromatic carbonyl groups, was revealed in the ^{13}C n.m.r. spectrum. Methylation (diazomethane) gave a heptamethyl ether (1b), m.p. 281—282 °C, which exhibited a molecular ion peak at *m/z* 568 in the mass spectrum. Further methylation of this product under more drastic conditions (dimethyl sulphate and potassium hydroxide, followed by treatment with diazomethane), yielded a non-crystalline undecamethyl ether (1c).

In the ^{1}H n.m.r. spectra of sanguisorbic acid dilactone and its derivatives (1b and c), one singlet and two *meta*-coupled aromatic proton signals (J 2 Hz) were observed, thus permitting the assignment of structure (1a). This assignment was supported by ^{13}C n.m.r. comparison with ellagic acid: the latter possesses a symmetrical structure and therefore shows only seven carbon signals, whereas sanguisorbic acid dilactone exhibits twenty-one clearly resolved carbon resonances, including two doublets and three hydroxy-bearing aromatic carbon singlets in the off-resonance spectrum due to an ' additional ' gallic acid residue.

Sanguisorbic acid dilactone is thus a gallic acid trimer and a structural isomer of valoneaic acid dilactone (5),[12] isolated by Schmidt and his co-workers from *Quercus valonea*.

Sanguin H-1 (2), a non-crystalline amorphous powder, $[\alpha]_D$ +95.8°, is very soluble in water and gave an intense blue colour with iron(III) chloride. On hydrolysis with tannase, it yielded gallic acid and ellagic acid along with glucose. The ^{1}H n.m.r. spectrum of sanguiin H-1 exhibited, in addition to the signals due to one hexahydroxydiphenoyl (δ 6.50 and 6.72) and two galloyl (δ 7.16 and 7.23) groups, an anomeric proton doublet (δ 6.59, J 4 Hz) which shifted downfield on acylation of the C(1) hydroxy-group. Furthermore, a triplet (δ 5.53, J 9 Hz), a double doublet (δ 5.25, J 4 and 9 Hz) and a two-proton broad singlet (δ 4.56) attributable to the ester-bearing protons were observed in the low field. These signals were assigned by means of spin-decoupling techniques. On irradiation at the frequency of the anomeric proton signal at δ 6.59, a double doublet at δ 5.25 changed into a doublet, thus permitting the assignment of this signal to the C(2) proton. A triplet at δ 5.53 could be assigned to the C(3) proton since this was



(1)
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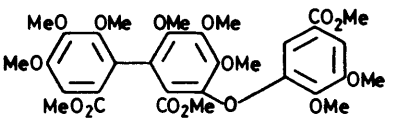
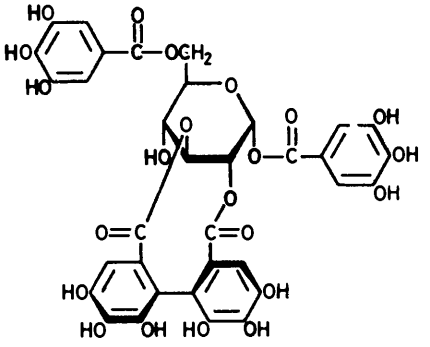
b; R = Me



(1c)



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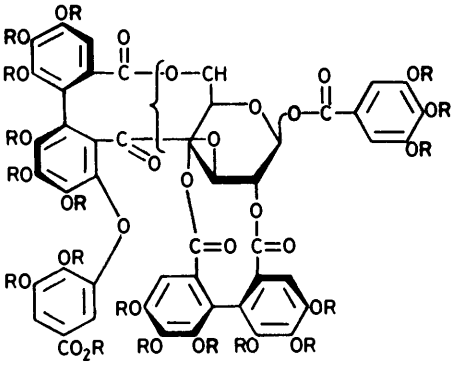

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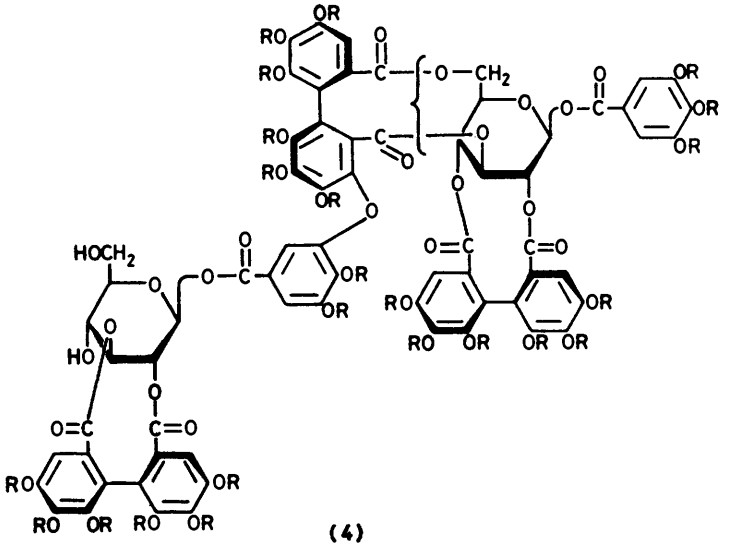

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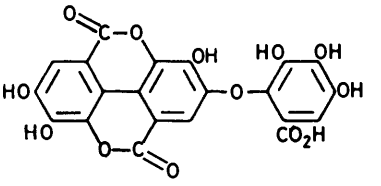
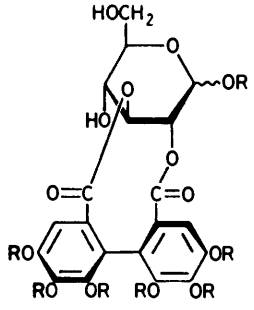


(5)

shown to be coupled with C(2) proton by irradiation of each signal in turn. A two-proton broad singlet at δ 4.56 was ascribable to the C(6) protons since its chemical shift was close to that of pentagalloyl glucose [6] and its signal shape did not change on irradiation at the frequency corresponding to the C(3) and C(4) protons. These facts indicated that the two galloyl and the hexahydroxydiphenoyl groups were located at C(1), C(2), and C(3) and C(6) in the glucose residue.

In order to allocate specifically these phenolic carboxylic acid groups within the glucose residue, partial hydrolysis with tannase was attempted.[7] Sanguiin H-1, as expected, quickly liberated gallic acid on addition of tannase, and the hydrolysate, after separation by chromatography over Sephadex LH-20, afforded a mixture of α- and β-anomers (6a), $[\alpha]_D$ +64.4°, the ^1H n.m.r. spectrum of which showed the presence of a hexahydroxydiphenoyl group (δ 6.66 and 6.74) and no galloyl peaks. ^{13}C N.m.r. analysis of the hydrolysate, although



(6)
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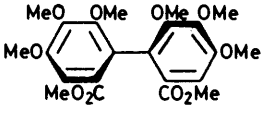
b; R = Me



(7)

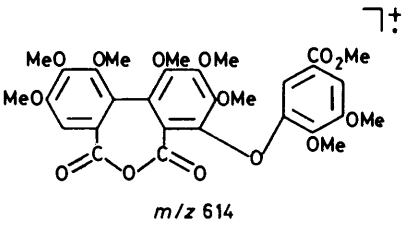
the signals were duplicated, permitted the assignment of structure (6a) on the grounds that the C(6) signals of the glucose moiety were shifted upfield by 1.6 p.p.m. as compared with sanguiin H-1; the lack of the *O*-galloyl group at C(1) caused duplication of the signals.

Accordingly, sanguiin H-1 was characterized as 1,6-digalloyl-2,3-(4,4′,5,5′,6,6′-hexahydroxydiphenoyl)-glucose.

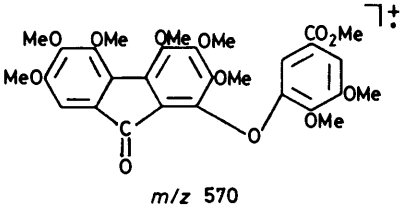
The chirality of the hexahydroxydiphenoyl group was determined as follows: the partial hydrolysate (6a) was methylated with dimethyl sulphate and potassium carbonate in acetone to give a mixture of anomeric heptamethyl ethers (6b), $[\alpha]_D$ −14.0°, which, on hydrolysis with aqueous alkali followed by methylation with diazomethane, afforded optically active (−)-dimethyl 4,4′,5,5′,6,6′-hexamethoxydiphenoate (7), $[\alpha]_D$ −23.7°;[13] thus the hexahydroxydiphenoyl group is in the *S*-series.

Sanguiin H-1 probably has an α-linkage at the anomeric centre of the glucose residue. Since it is known that pedunculagin [2,3;4,6-bis(hexahydroxydiphenoyl)-glucose] [14] and eugeniin [1,2,3-trigalloyl-4,6-(hexahydroxydiphenoyl)glucose] [7] have the $^4C_1$ conformation, the small coupling constant (*J* 4 Hz) of the anomeric proton signal and notable upfield shift (90.5 p.p.m.) of the C(1) carbon signal are only explicable if the C(1) galloyl group is axially oriented.

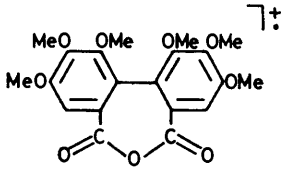
Sanguiin H-2 (3a), a tan amorphous powder, $[\alpha]_D$ +7.9°, $C_{48}H_{32}O_{31}$,$5H_2O$, gave a red colour characteristic of ellagitannins with Na_2SO_3–Na_2CO_3 reagent.[15] Methylation with dimethyl sulphate and potassium carbonate in dry acetone yielded a crystalline heptadecamethyl ether



m/z 614

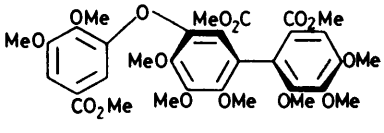


m/z 570



m/z 404

(3b), m.p. 153—155 °C, $[\alpha]_D$ −30.2°, whose mass spectrum suggested the presence of a sanguisorboyl (*m/z* 570, 614), a hexahydroxydiphenoyl (*m/z* 404, 360), and a galloyl (*m/z* 212, 195) group in sanguiin H-2. This methyl ether was subsequently hydrolysed with aqueous alkali, and the acidic products, after methylation with diazomethane, were separated by silica gel chromatography giving methyl 3,4,5-trimethoxybenzoate, and the optically active carboxylic acid esters (7), $[\alpha]_D$ −27.4°,

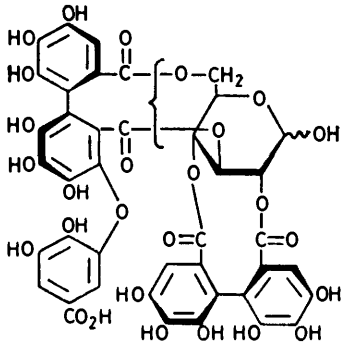


(1c′)

and (1c′), $[\alpha]_D$ −43.7°. From the neutral fraction of the alkaline hydrolysis products, glucose was detected by paper chromatography. The chirality of the ester (1c′) was established as *R* by analysis of the c.d. spectrum, which showed a negative plain curve at 244 nm and a positive one at 265 nm, opposite to those of the ester (7) with the *S*-configuration.

On the basis of these results, in combination with the

^{1}H n.m.r. data which revealed seven aromatic protons, sanguiin H-2 was deduced to contain one galloyl, one hexahydroxydiphenoyl, and one sanguisorboyl group, joined to glucose through ester linkages. The possibility that the carbonyl group of the ' additional ' gallic acid residue in the sanguisorboyl group was also linked to the glucose moiety was eliminated by the appearance of fragment ions at *m/z* 570 and 614 in the mass spectrum of the heptadecamethyl ether.



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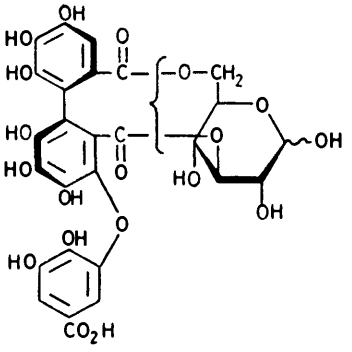
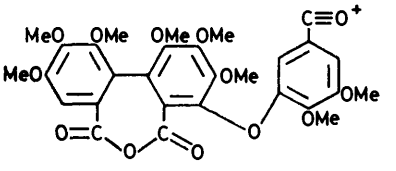


(9)

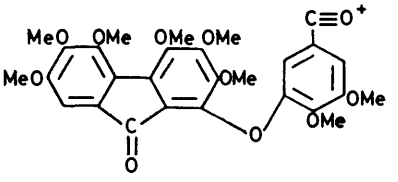
Hydrolysis of sanguiin H-2 with tannase as for sanguiin H-1 gave gallic acid, ellagic acid, and two partial hydrolysates, (8) and (9). The hydrolysate (8) was easily identified as 1-degalloylsanguiin H-2 on the basis of ^{1}H n.m.r. analysis: a two-proton singlet due to the galloyl group was absent, and the spectrum was complicated by the presence of α- and β-anomers. On the other hand, the hydrolysate (9) contained only a sanguisorboyl group, as revealed by three aromatic proton signals. Of the complex glucose proton signals, a double doublet at δ 5.11 (*J* 6 and 14 Hz) assignable to one of the C(6) protons is notable. Since this spectrum was quite unlike that of 4,6-(hexahydroxydiphenoyl)-glucose, obtained by partial hydrolysis of eugeniin,[7] the sanguisorboyl group could be located at positions C(6) and C(3) in the glucose moiety. The assignment of the structure (9) was also supported by comparison of the C(6) and C(3) proton chemical shifts with those of corilagin [1-galloyl-3,6-(hexahydroxydiphenoyl)glucose].[16]

Consequently, sanguiin H-2 was assigned the structure (3a).

Sanguiin H-3 (4a), a tan amorphous powder, $[\alpha]_D$ +63.2°, $C_{68}H_{48}O_{44}$,$3H_2O$, was obtained from the aqueous layer after extraction with ethyl acetate, and, like sanguiin H-2, formed a red pigment with Na_2CO_3–Na_2SO_3. Acid hydrolysis yielded gallic acid and glucose, together with ellagic acid and sanguisorbic acid dilactone, identified respectively as their tetra- and hepta-methyl ethers. The existence of these phenolic carboxylic acid residues in sanguiin H-3 was supported by fragment ion peaks at *m/z* 195, 360, 404, 539 and 583 in the mass spectrum of its tricosamethyl ether (4b), m.p. 190—192 °C. These characteristics closely resemble those of sanguiin H-2. The ^{1}H n.m.r. spectrum

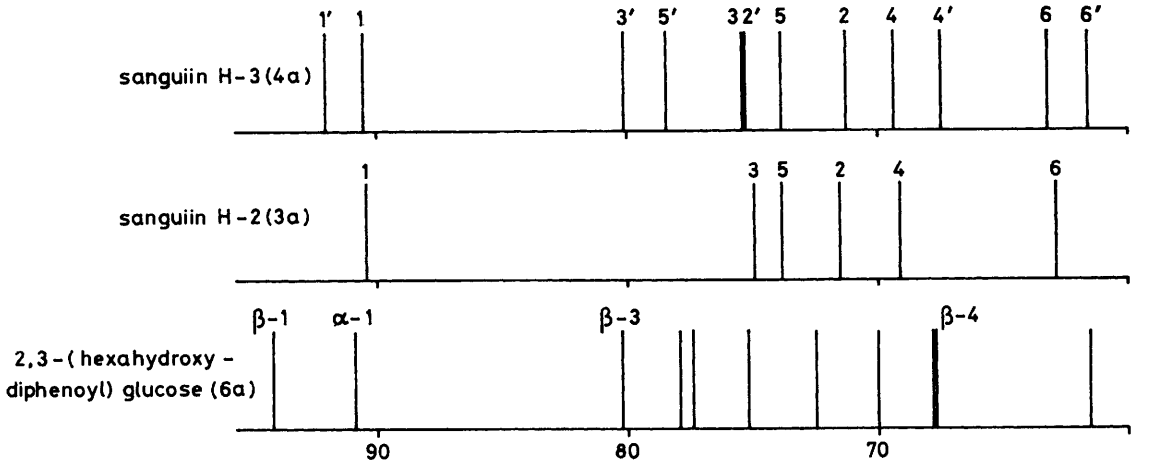


m/z 583



m/z 539

of sanguiin H-3 showed nine aromatic proton signals of which seven corresponded to those in sanguiin H-2. The remaining two signals, δ 6.21 and 6.68, are probably due to an additional hexahydroxydiphenoyl group. Acetylation of the tricosamethyl ether (4b) afforded the diacetate (4c), m.p. 187—189 °C, which showed a singlet at δ 2.13 due to two acetyl groups in the ^{1}H n.m.r. spectrum. The use of field-desorption mass spectroscopy for this methyl ether diacetate (4c) confirmed its molecular weight; the spectrum was notable for the appearance of very intense peaks at *m/z* 1974 $(M)^+$ and 1 997 $(M + Na)^+$. In order to determine the positions of the free hydroxy-groups in glucose moiety, the tricosamethyl ether (4b) was further methylated with methyl iodide and silver oxide in dimethylformamide,[17] and the permethyl ether (4d), m.p. 198—201 °C, $[\alpha]_D$ −71.8°, thus obtained was subjected to methanolysis. The resulting methyl glycoside was analysed by g.l.c. to confirm its identification as methyl 4,6-di-*O*-methyl-glucopyranoside. On the basis of this result the free hydroxy-groups must be at C(4) and C(6). From these facts, coupled with the presence of two anomeric protons (δ 6.15 and 6.53) and twelve sugar carbon signals (Figure), sanguiin H-3 is considered to contain sanguiin H-2 and 2,3-(hexahydroxydiphenoyl)glucose residues.



^{13}C Chemical shifts of sugar carbon atoms in sanguiins H-3 and H-2, and 2,3-(hexahydroxydiphenoyl)glucose (solvent CD_3COCD_3; standard $SiMe_4$)

Partial hydrolysis of sanguiin H-3 with tannase or acid yielded no useful information, owing to the formation of a complex mixture. However, refluxing in aqueous solution selectively cleaved the linkage between the two units to yield two hydrolysates shown to be 2,3-(hexahydroxydiphenoyl)glucose (6a) and sanguiin H-2 (3a).

Accordingly, sanguiin H-3 was assigned the formula (4a), a novel dimeric structure containing two glucose cores.

EXPERIMENTAL

I.r. spectra were obtained with a JASCO IR-G spectrometer. Electron-impact and field-desorption mass spectra were taken with JEOL JMS-01SG and JMS-DX 300 instruments, respectively. ^1H N.m.r. and ^{13}C n.m.r. spectra were recorded on JEOL PS-100 and Jeol FX-100 spectrometers, with $SiMe_4$ as internal standard, and c.d. data were obtained with a JASCO J-20C spectropolarimeter. T.l.c. was performed on precoated Kieselgel 60 F_{254} plates (Merck; 0.20 mm) and precoated cellulose F_{254} plates (Merck; 0.10 mm), and spots were detected by use of iron(III) chloride and anisaldehyde–sulphuric acid reagent sprays. Column chromatography was carried out with Sephadex LH-20 (25—100μ; Pharmacia Fine Chemicals), Kieselgel 60 (Merck; 70—230 mesh) and Avicel microcrystalline cellulose (Funakoshi). Analytical g.l.c. for methyl sugars was conducted over 1% neopentyl glycol succinate polyester with nitrogen as carrier gas.

Isolation of Tannins.—Freshly collected underground parts (2.8 kg) of *Sanguisorba officinalis* were extracted at room temperature with acetone–water (4 : 1). The acetone was removed by evaporation under reduced pressure (*ca.* 40 °C); the aqueous solution then deposited a crystalline saponin mixture which gave a positive reaction to the Liebermann–Burchard test. The filtrate was extracted with ethyl acetate to give fraction (93.5 g) containing low-molecular phenolics. Chromatography of this fraction on a Sephadex LH-20 column (48 × 5.0 cm) eluted with ethanol yielded (+)-catechin (18 g), (±)-gallocatechin (0.8 g), gallic acid (1.0 g), ellagic acid (0.3 g), and procyanidins B-3 (0.5 g) and C-2 (0.06 g).[11] Further elution of this column with ethanol–water (9 : 1)[6] gave a fraction containing sanguiin H-1 and eugeniin. These compounds were separated by chromatography over a cellulose column using 2% acetic acid to afford chromatographically pure sanguiin H-1 (0.19 g) and eugeniin (1.5 g). The Sephadex LH-20 column was subsequently eluted with ethanol–water–acetone (54 : 36 : 10)[6] to give sanguiin H-2 (0.5 g). The aqueous layer remaining after the ethyl acetate extraction was mixed with Celite-545 (1 kg) and air-dried. A brown powder thus obtained was packed in a glass column and eluted with acetone. Evaporation of the eluate afforded crystalline sanguisorbic acid dilactone (0.6 g). The filtrate was evaporated to dryness and the residue was chromatographed over Sephadex LH-20 in ethanol–water–acetone (54 : 36 : 10) to give sanguiin H-3 (4.0 g).

Sanguisorbic acid dilactone (1a), recrystallized from chloroform–methanol, had m.p. >300 °C (Found: C, 48.3; H, 2.85. $C_{21}H_{10}O_{13},3H_2O$ requires C, 48.1; H, 3.05%); $\nu_{max.}$ (KBr) 3 400 (OH), 1 720 ($ArCO_2$), 1 700 ($ArCO_2H$), and 1 610 cm^{-1} (Ar); δ_H (C_2D_6SO) 6.50, 7.12 (each 1 H, d, *J* 2 Hz, aromatic), and 7.50 (1 H, s, aromatic); δ_C (C_2D_6SO) 101.4, 105.5 (C-2″, C-6″), 108.0 (C-3), 109.9, 110.7, 111.4, 111.6 (C-1, C-1′, C-2, C-2″), 119.5 (C-1″), 133.7 (C-5′), 136.2 (C-5), 138.6, 138.9 (C-4, C-6), 139.3 (C-6), 140.6, 141.0 (C-3′, C-4″), 145.3, 146.1, 147.7 (C-4, C-3″, C-5″), 155.0, 158.5 (CO_2), and 166.7 (CO_2H). A suspension of sanguisorbic acid dilactone (30 mg) in acetone was treated overnight with ethereal diazomethane. Crystallization of the product with chloroform–acetone afforded the *heptamethyl ether* (1b) as a white powder, m.p. 281—282 °C (Found: C, 58.2; H, 4.2. $C_{28}H_{24}O_{13}$ requires C, 58.25; H, 4.35%); $\nu_{max.}$ (KBr) 1 750 ($ArCO_2$) and 1 700 cm^{-1} ($ArCO_2H$); *m/z* 568 $(M)^+$ and 537 $(M - OCH_3)^+$; δ_H ($CDCl_3$) 3.76, 3.88, 3.95, 4.03, 4.10, 4.17, 4.28 (each 3 H, s, OCH_3), 6.81, 7.34 (each 1 H, d, *J* 2 Hz, H-2″, H-6″), and 7.68 (1 H, s, H-3). A solution of the heptamethyl ether (1c) (100 mg) in aqueous 3M-sodium hydroxide (3 ml) was heated for 0.5 h with dimethyl sulphate (1 ml). After cooling, the mixture was neutralized with 3M-sulphuric acid, and extracted with ether. The organic layer was washed with water, dried (Na_2SO_4), and evaporated and the oily residue was treated for 1 h with ethereal diazomethane. Purification over silica gel (benzene–acetone) afforded *trimethyl octa-O-methylsanguisorboate* (1c) (86 mg) as a

colourless syrup (Found: C, 57.8; H, 5.55. $C_{32}H_{36}O_{15}$ requires C, 58.2; H, 5.5%); $\nu_{max.}$ (KBr) 1 720 ($ArCO_2$) and 1 590 cm^{-1} (Ar); δ_H ($CDCl_3$) 3.68—3.98 (33 H, OCH_3 × 11), 7.13, 7.31 (each 1 H, d, *J* 2 Hz, H-2″, H-6″), and 7.33 (1 H, s, H-3).

Sanguiin H-1 (2a), obtained as a tan amorphous powder, had $[\alpha]_D$ +93.5° (*c* 0.7, in acetone); δ_H (CD_3COCD_3) 4.04—4.28 (2 H, m, H-4 and H-5), 4.56 (2 H, brs, H-6), 5.25 (1 H, dd, *J* 9 and 4 Hz, H-2), 5.53 (1 H, t, *J* 9 Hz, H-3), 6.50 (1 H, s, H of HHDP *), 6.59 (1 H, d, *J* 4 Hz, H-1), 6.72 (1 H, s, H of HHDP), and 7.16, 7.23 (each 2 H, s, galloyl H); δ_C (CD_3COCD_3) 63.1 (C-6), 67.6 (C-4), 73.5 (C-2 and C-5), 77.8 (C-3), 90.5 (C-1), 107.0, 107.5 (HHDP C-3, C-3′), 109.7, 110.0 (galloyl C-2, C-6), 114.2 (HHDP C-1, C-1′), 120.1, 121.1 (galloyl C-4), 144.0, 144.8 (HHDP C-4, C-4′, C-6 and C-6′), 145.7, 145.8 (galloyl C-3, C-5), and 164.6, 166.3, 168.3, 169.2 (CO_2).

A solution of sanguiin H-1 (10 mg) in water was incubated overnight with tannase at 37 °C. The products were analysed directly by cellulose t.l.c. using 2% AcOH and butanol–acetic acid–water (6 : 1 : 2) for phenolic acids, and butanol-pyridine–water (6 : 4 : 3) for sugars. Gallic acid, ellagic acid, and glucose were identified by co-chromatography with authentic samples.

A solution of sanguiin H-1 (105 mg) in water was shaken for 2 h with tannase at 37 °C. Evaporation under reduced pressure left a residue, which was treated with ethanol. The ethanol-soluble portion was applied to a Sephadex LH-20 column (11.0 × 3.0 cm) in ethanol to give gallic acid and 2,3-(hexahydroxydiphenoyl)glucose (6a) (50 mg) as a tan amorphous powder, $[\alpha]_D$ +64.6° (*c* 0.8, in MeOH); δ_H (CD_3COCD_3–D_2O) 3.60—5.14 (m), 5.39 (t, *J* 9 Hz, H-3), 5.42 (d, *J* 4 Hz, H-1), and 6.66, 6.74 (each 1 H, s, HHDP).

Methyl 2,3-(hexamethoxydiphenoyl)glucoside (6b). A mixture of 2,3-(hexahydroxydiphenoyl)glucose (6a) (31 mg), anhydrous potassium carbonate (0.8 g), and dimethyl sulphate (0.5 ml) in dry acetone was refluxed for 2.5 h with stirring. After removal of inorganic salts, the filtrate was concentrated to a syrup, which was chromatographed over silica gel using benzene–acetone (7 : 3) to give methyl 2,3-(hexamethoxydiphenoyl)glucoside (6b) (21 mg) as a white amorphous powder, $[\alpha]_D$ −14.0° (*c* 0.2, in acetone); δ_H ($CDCl_3$) 3.54—4.10 (25 H, OCH_3 H-4, H-5, and H-6), 4.56—5.52 (3 H, m, H-1, H-2, and H-3), and 6.75, 6.80 (each 1 H, s, HHDP).

Dimethyl (−)-hexamethoxydiphenoate (7). A solution of methyl 2,3-(hexamethoxydiphenoyl)glucoside (6b) (11 mg in aqueous 10% sodium hydroxide was heated for 0.5 h at 90 °C. The mixture, after cooling, was acidified with 12% hydrochloric acid, and the resulting precipitates were extracted with ether. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to yield a syrup, which was methylated for 15 min with ethereal diazomethane. The crude methyl ethers were purified by p.l.c. using benzene–ether (3 : 2) to give dimethyl hexamethoxydiphenoate (7) as a colourless syrup, $[\alpha]_D$ −23.7° (*c* 0.5, in $CHCl_3$).

Sanguiin H-2 (3a), obtained as a tan amorphous powder, had $[\alpha]_D$ +7.9° (c 0.98, in acetone) (Found: C, 48.4; H, 3.85. $C_{48}H_{32}O_{31}$,$5H_2O$ requires C, 48.25; H, 3.55%); δ_H (CD_3COCD_3) 3.75 (1 H, br, s, *J* 13 Hz, H-6), 3.88 (1 H, m, H-5), 4.80—4.98 (2 H, m, H-3 and H-4), 5.24 (1 H, m, H-2), 5.42 (1 H, dd, *J* 13 and 6 Hz, H-6), 6.37, 6.39 (each 1 H, s, H of HHDP), 6.52 (1 H, d, *J* 4 Hz, H-1), 6.70 (1 H, s, aromatic), 7.08 (2 H, s, galloyl), and 7.12, 7.27 (each 1 H, d, aromatic); δ_C (CD_3COCD_3) 164.6, 165.5, 167.7, 168.1, and 168.9 (COO). A mixture of sanguiin H-2 (30 mg), anhydrous K_2CO_3 (2.5 g), and dimethyl sulphate (1.5 ml) in dry acetone (25 ml) was refluxed for 3 h with stirring. Work-up as for methyl 2,3-(hexamethoxydiphenoyl)glucoside (6b) gave the *heptadecamethyl ether* (3b) as a colourless powder (from EtOH), m.p. 153—155 °C, $[\alpha]_D$ −30.2° (*c* 0.9, in acetone) (Found: C, 58.25; H, 5.2. $C_{66}H_{68}O_{31}$ requires C, 58.4; H, 5.05%); δ_H ($CDCl_3$) 3.40—4.05 (OCH_3), 4.46 (1 H, m, H-5), 4.98—5.38 (3 H, m, H-2, H-3, and H-4), 5.63 (1 H, dd, *J* 14 and 7 Hz, H-6), 6.39 (1 H, br, s, H-1), and 6.41 (2 H, s, aromatic). A solution of the heptadecamethyl ether (3b) in aqueous 10% sodium hydroxide (10 ml) and methanol (0.5 ml) was heated on a boiling water-bath for 2.5 h. The mixture was acidified with 12% hydrochloric acid and extracted with ether. The extract, after removal of the solvent, was treated for 30 min with ethereal diazomethane. The crude methylation products were chromatographed over silica gel using benzene–ether to afford methyl 3,4,5-trimethoxybenzoate (10 mg), m.p. 80—81 °C, dimethyl hexamethoxydiphenoate (7) (18 mg), $[\alpha]_D$ −27.4° (*c* 0.57, in $CHCl_3$), and *trimethyl octa-O-methylsanguisorboate* (1c′) (16 mg), $[\alpha]_D$ −43.7° (*c* 0.35, in $CHCl_3$); c.d. (MeOH) $[\theta]_{298}$ +3.3 × 10^5, $[\theta]_{265}$ +19.8 × 10^5, $[\theta]_{254}$ 0, $[\theta]_{244}$ −3.3 × 10^6. The foregoing acidic solution, after extraction with ether, was treated with Amberlite IRA-400 (OH^- form) and Dowex 50W-X8 (H^+ form) resins, and the neutral solution was evaporated to a syrup, which was analysed by cellulose t.l.c. using butanol–pyridine–water (6 : 4 : 3). Only a spot for glucose was detected on spraying with aniline hydrogen phthalate reagent.

A solution of sanguiin H-2 (120 mg) in water was incubated for 28 h with tannase at 40 °C. The mixture was treated as before to give a hydrolysate mixture which was separated by chromatography over Sephadex LH-20. Elution with ethanol gave gallic acid and ellagic acid. Elution with ethanol–water (4 : 1) gave 3,6-*(sanguisorboyl)-glucose* (9) (4 mg) as a tan amorphous powder, δ_H (CD_3-$COCD_3$–D_2O) 5.07 (1 H, m, H-3), 5.11 (1 H, dd, *J* 6 and 14 Hz, H-6), 6.65 (1 H, d, *J* 2 Hz, aromatic), 6.92 (1 H, br, s, aromatic), and 7.23 (1 h, d, *J* 2 Hz, aromatic). Elution with ethanol–water (7 : 3) yielded 1-*degalloylsanguin* H-2 (8) (11 mg) as a tan amorphous powder, δ_H (CD_3COCD_3–D_2O) 5.46 (1 H, dd, *J* 8 and 14 Hz, H-6), 6.33, 6.55 (each 1 H, br, s, aromatic), 6.72, 6.75 (1 H in total, s, aromatic), and 7.08, 7.16 (each 1 H, d, *J* 2 Hz, aromatic).

Sanguiin H-3 (4a), obtained as a tan amorphous powder, had $[\alpha]_D$ +63.2° (*c* 0.7, in acetone) (Found: C, 50.8; H, 3.8. $C_{68}H_{48}O_{44}$,$3H_2O$ requires C, 50.3; H, 3.35%); δ_H (CD_3-$COCD_3$) 3.60—4.23, 4.84—5.52 (sugar H), 6.15 (1 H, d, *J* 8 Hz, anomeric H), 6.21 (1 H, aromatic), 6.39 6.42 (each 1 H, s, aromatic), 6.53 (1 H, d, *J* 13 Hz, anomeric H), 6.68, 6.70 (each 1 H, s, aromatic), 7.03 (1 H, d, *J* 2 Hz, sanguisorboyl), 7.16 (2 H, s, galloyl), and 7.20 (1 H, d, *J* 2 Hz, sanguisorboyl); δ_C (CD_3COCD_3), 107.2, 107.9 (HHDP C-3, C-3′), 110.2 (galloyl C-2, C-6), 125.7, 126.1, 126.7 (HHDC C-2, C-2′), and 164.5, 164.9, 165.2, 167.7, 167.9, 168.0, 169.1 (CO_2). A solution of sanguiin H-3 (4a) in N-sulphuric acid was refluxed for 7.5 h. The resulting precipitates, after cooling, were filtered off to afford a pale brown powder, which was methylated overnight with ethereal diazomethane. The methylation products were separated by p.l.c. using benzene–acetone (4 : 1). Elution of the band at R_F 0.45—0.50 gave sanguisorbic acid

* HHDP = hexahydroxydiphenoyl.

dilactone heptamethyl ether (1b) (26 mg), m.p. 281—282 °C. Elution of the band at R_F 0.50—0.57 gave ellagic acid tetramethyl ether (30 mg), m.p. >300 °C; $\nu_{max.}$ (KBr) 2 950, 2 850, 1 735, 1 610, 1 570, 1 490, and 1 360 cm^{-1}. Extraction of the foregoing filtrate with ethyl acetate yielded gallic acid (25 mg). The aqueous layer was neutralized with barium carbonate, and the resulting inorganic salts were filtered off. The filtrate was evaporated to dryness, and the residue in methanol was applied to a Sephadex LH-20 column to give glucose as a colourless syrup (50 mg).

A mixture of sanguiin H-3 (1.05 g), anhydrous potassium carbonate (12 g), and dimethyl sulphate (8 ml) in dry acetone (60 ml) was refluxed for 3 h. Work-up as before gave the *tricosamethyl ether* (4b) (432 mg) as a white powder, m.p. 210—213 °C, $[\alpha]_D$ −34.7° (*c* 0.48, in acetone) (Found: C, 57.05, H, 5.05. $C_{93}H_{98}O_{44},2H_2O$ requires C, 57.1; H, 5.25%); *m/z* 614, 583, 570, 539, 422, 404, 360, 345, 330, 212, and 195; δ_H ($CDCl_3$) 5.45 (1 H, dd, *J* 14 and 8 Hz, H-6), 5.94 (1 H, d, *J* 7 Hz, H-1′), 6.36 (1 H, br, s, H-1), 6.43 (2 H, s, aromatic), 6.51, 6.78, 6.93 (each 1 H, s, aromatic), 6.95 (1 H, d, *J* 2 Hz, aromatic), and 7.16 (3 H, m, aromatic); δ_C ($CDCl_3$) 55.6, 56.0 (OCH_3), 60.8 (C-6′), 62.4 (C-6), 66.5 (C-4′), 68.5 (C-4), 70.1 (C-2), 72.5 (C-5), 73.0, 74.9 (C-3, C-5′), 77.5 (C-2′), 79.3 (C-3′), 90.9 (C-1), and 91.5 (C-1′).

The tricosamethyl ether (4b) (11 mg) was acetylated overnight with acetic anhydride (0.5 ml) and dry pyridine (0.2 ml). Usual work-up, followed by crystallization from ethanol, afforded the *tricosamethyl ether diacetate* (4c) as a white powder, m.p. 195—197 °C (Found: C, 57.35; H, 5.0. $C_{95}H_{98}O_{46}$ requires C, 57.75; H, 5.0%); *m/z* 1 998 (M + H + Na)$^+$, 1 997 (M + Na)$^+$, 1 975 (M + H)$^+$, 1 974 (M)$^+$, 1 795 (M + H − 180)$^+$, 987 (M)$^{2+}$, 1 341, and 663; δ_H ($CDCl_3$) 2.13 (6 H, s, 2 × Ac), 5.98 (1 H, d, *J* 7 Hz, H-1′), 6.35 (2 H, s, aromatic), 6.37 (1 H, br, s, H-1), 6.53, 6.56, 6.94 (each 1 H, s, aromatic), and 7.12 (4 H, br, s, aromatic).

A mixture of the tricosamethyl ether (4b) (61 mg), methyl iodide (2.5 ml), and freshly prepared silver oxide (1.2 g) in dimethylformamide (1.5 ml) was stirred for 1 h at room temperature. Filtration, and evaporation of the filtrate under a stream of nitrogen gave an oil, which was diluted with water and extracted with ether. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to give a crude methyl ether. Purification on a silica gel column using benzene–acetone (23 : 2) yielded the *pentacosamethyl ether* (4d) (26 mg) as a white powder, m.p. 198—201 °C, $[\alpha]_D$ −71.8° (*c* 0.17, in $CHCl_3$); ν_{max} (KBr) 1 758, 1 735, 1 720 ($ArCO_2$), and 1 580 cm^{-1} (Ar); δ_H ($CDCl_3$) 6.06 (1 H, d, *J* 7 Hz, H-1″), 6.56 (1 H, br, s, H-1), and 6.54—7.29 (9 H, s, aromatic).

A solution of the pentacosamethyl ether (4d) (10 mg) in N-NaOH (methanol–water 1 : 1) was refluxed for 30 min. The mixture, after neutralization with Dowex 50W-X8 (H^+ form), was evaporated to give an oil, which was methanolysed for 30 min with methanolic N-hydrochloric acid. Neutralization with Amberlite IRA-400 (OH^- form) and evaporated afforded a colourless syrup. Purification on a silica gel column using ethyl acetate–methanol (50 : 1) afforded methyl 4,6-di-*O*-methylglucopyranoside (g.l.c. t_R 4.09 min; column temp. 160 °C; flow rate 40 ml min^{-1}).

A solution of the tricosamethyl ether (4b) (93 mg) in 10% NaOH (methanol–water 3 : 5) was refluxed for 3 h. The mixture was acidified with 12% hydrochloric acid, and extracted with ether. The organic layer was evaporated and the residue was treated for 30 min with ethereal diazomethane to give crude methyl ethers, which were separated by silica gel chromatography using benzene–ether (85 : 15—80 : 20) to give methyl trimethoxybenzoate (12 mg), m.p. 80—81 °C; dimethyl hexamethoxydiphenoate (7) (38 mg), $[\alpha]_D$ −28.8° (*c* 0.38, in $CHCl_3$); and trimethyl octa-*O*-methylsanguisorboate (1c′) (32 mg), $[\alpha]_D$ −43.6° (*c* 0.46, in $CHCl_3$).

A solution of sanguiin H-3 (480 mg) in water (30 ml) was refluxed for 42 h, then evaporated. The residue was chromatographed over Sephadex LH-20. Elution with ethanol afforded gallic acid and 2,3-(hexahydroxydiphenoyl)-glucose (58 mg). Further elution with ethanol–water–acetone (54 : 36 : 10) yielded sanguiin H-2 (67 mg). A large amount of starting material was recovered by subsequent elution with ethanol–water–acetone (54 : 36 : 10).

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