1342. *Gallotannins. Part XII.*[1] *Phenolic Constituents of* Arctostaphylos uva-ursi *L. Spreng*

By G. Britton and E. Haslam

The tannin from the leaves of the mountain bearberry is a penta- to hexa-*O*-galloyl-β D-glucose and probably represents a prototype of the more complex gallotannins. Three galloyl esters of arbutin isolated from the same source were assigned structures and the possible biogenetic relationship of 2 *O*-galloylarbutin to the *C*-glucoside bergenin is commented upon.

Members of the plant family *Ericaceae* produce a notable diversity of secondary metabolites; pre-eminent amongst these is arbutin [*p*-hydroxyphenyl β-D-glucoside (Ia)] and in earlier times its presence led to the use of extracts of this plant family in medical treatment.[2] Extracts of the leaves of the mountain bearberry (*Arctostaphylos uva-ursi*) have

[1] Part XI, P. N. Crabtree, E. Haslam, R. D. Haworth, S. D. Mills, and J. E. Stangroom, *J.*, 1965. 6888.

[2] H. Kreitmar, *Pharmazie*, 1953, **8**, 346.

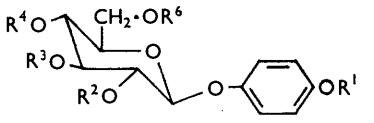
also found use as tanning agents [3] (Gayuba) and in this work a detailed study of the phenolic compounds produced by this plant has been made.

Paper-chromatographic examination of the phenolic extract from the leaves of *Arctostaphylos uva-ursi* showed the presence of at least 20 components. In addition to arbutin three substances (A, B, C) were identified as *p*-hydroxyphenyl β-D-glucoside derivatives by means of their distinctive blue coloration with Gibbs reagent.[4] Several flavonoid glycosides [identified [5] after acid hydrolysis as derivatives of quercetin (II; R = H) and myrecitin (II; R = OH), in agreement with earlier work [6,7]] were shown to be present but the major phenolic components of the extract (A—G) all displayed the characteristic colour reactions associated with esters of gallic acid. The predominant compound (D) in this group, approximated on analysis [8] to a penta- to hexa-*O*-galloyl-D-glucose derivative (molecular weight 980) and corresponds presumably to the tannin previously described by Perkin [6] and by Herrmann.[9] Since it gave ellagic acid (III) on hydrolysis Herrmann [9] suggested a relationship to Turkish gallotannin [10,11] but this observation may have resulted from its contamination with other substances (E, F) in the extract which have been tentatively identified in this work as ellagitannins and one (E) more specifically as corilagin [12,13] (1-*O*-galloyl-3,6-di-*O*-hexahydroxydiphenoyl-β-D-glucose). Methanolysis [8] of the tannin gave methyl gallate and a mixture (5 : 1) of β-penta-*O*-galloyl-D-glucose (IVa) and 2,3,4,6-tetra-*O*-galloyl-D-glucose (IVb) whose structures were confirmed by methods previously described.[8] At no stage during the solvolysis was the depside methyl *m*-digallate detected and this observation suggests that the tannin consists predominantly of a β-penta-*O*-galloyl-D-glucose structure to which is attached *one* galloyl group in depsidic fashion. Attempts to show whether this galloyl group is attached randomly or at one specific position have, as yet, been unsuccessful. Thus, although related infrared spectral studies of *o*-hydroxydepsides in aprotic media [14,15] show the *o*-hydroxyl group to be hydrogen bonded to one or other of the oxygen atoms in the ester function, attempted partial alkylation of the tannin with diazomethane and diphenyldiazomethane, leaving the depside still susceptible to ready solvolysis, failed. Further work on this problem is in progress since its solution should, by extrapolation, allow clarification of the structures of the related and more complex tannins (Chinese, Sumach, and Turkish) [8,11] in particular in relation to the disposition of the depsidically linked galloyl groups.[16]

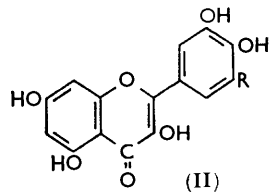
Compounds A, B, and C—present to the extent of 0·05% of the dry weight of the extract—all gave glucose, gallic acid, and quinol on acid hydrolysis, and on treatment with tannase [17] or dilute ammonia gave arbutin (Ia) and gallic acid thus permitting their formulation as mono-*O*-galloyl derivatives of arbutin. Partial acid hydrolysis of A or B gave quinol and substances, showing a positive reaction to aniline hydrogen phthalate,[18] which were identified paper chromatographically as mono-*O*-galloyl-D-glucose derivatives. Compound A underwent an unusually ready rearrangement at 0° in dilute ammonia to give compound B which was relatively stable to this treatment and in this respect A resembled

[3] A. Y. Gil and J. T. Ochoa, *Inst. forestal invest. v experiencias* (*Madrid*), 1955, **26**, 1.
[4] G. Britton, E. Haslam, and M. O. Naumann, *J.*, 1965, 5649.
[5] J. B. Harborne, *J. Chromatography*, 1959, **2**, 581.
[6] A. G. Perkin, *J.*, 1900, 424.
[7] R. Kawagati, K. W. Kim, and J. Matusita, *J. Pharm. Soc. Japan*, 1959, **59**, 50.
[8] R. Armitage, G. S. Bayliss, J. W. Gramshaw, E. Haslam, R. D. Haworth, K. Jones, H. J. Rogers, and T. Searle, *J.*, 1961, 1842.
[9] K. Herrmann, *Arch. Pharm.*, 1953, **286**, 515.
[10] K. Freudenberg, "Tannin, Cellulose and Lignin," Verlag Chemie, Berlin, 1933.
[11] R. Armitage, E. Haslam, R. D. Haworth, and T. Searle, *J.*, 1962, 3808.
[12] O. Th. Schmidt, *Fortschr. Chem. org. Naturstoffe*, 1956, **14**, 71.
[13] W. E. Hillis and A. Carle, *Biochem. J.*, 1962, **82**, 435.
[14] R. Biggins, T. Cairns, G. Eglinton, E. Haslam, and R. D. Haworth, *J.*, 1963, 1750.
[15] R. Biggins and E. Haslam, *J.*, 1965, 6883.
[16] E. Haslam and R. D. Haworth, "Progress in Organic Chemistry," vol. 6, Butterworths, London, 1964.
[17] E. Haslam, R. D. Haworth, K. Jones, and H. J. Rogers, *J.*, 1961, 1829.
[18] L. Hough, *J.*, 1950, 1702.

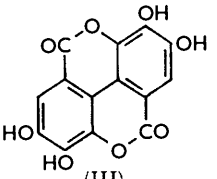
closely the behaviour of 2-*O*-caffeoylarbutin (Ib), recently isolated [4] from *Vaccinium vitis idaea*, another member of the *Ericaceae* plant family. Compound A is therefore provisionally regarded as 2-*O*-galloylarbutin (Ic) but this assignment awaits confirmation by synthesis. Compound B analogously in view of its relative stability to ammonia at 0° was assumed to



(Ia: $R^1 = R^2 = R^3 = R^4 = R^6 = H$)
[Ib: $R^1 = R^3 = R^4 = R^6 = H$, $R^2 = CO{\cdot}CH{:}CH{\cdot}C_6H_3(OH)_2$-3,4]
[Ic: $R^1 = R^3 = R^4 = R^6 = H$, $R^2 = CO{\cdot}C_6H_2(OH)_3$-3,4,5]
(Id: $R^1 = R^2 = R^3 = R^4 = H$, $R^6 = CO{\cdot}C_6H_2(OH)_3$-3,4,5]
(Ie: $R^1 = R^2 = R^3 = R^4 = CO{\cdot}Me$, $R^6 = H$)
(If: $R^1 = CH_2{\cdot}C_6H_5$, $R^2 = R^3 = R^4 = R^6 = H$)
[Ig: $R^1 = CO{\cdot}C_6H_2(OH)_3$-3,4,5, $R^2 = R^3 = R^4 = R^6 = H$]
(Ih: $R^1 = H$, $R^2 = R^3 = R^4 = R^6 = CO{\cdot}Me$)
[Ii: $R^1 = CO{\cdot}C_6H_2(OH)_3$-3,4,5, $R^2 = R^3 = R^4 = H$, $R^6 = CO{\cdot}Me$]



(II)



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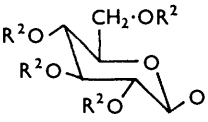
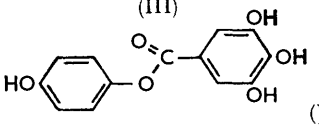
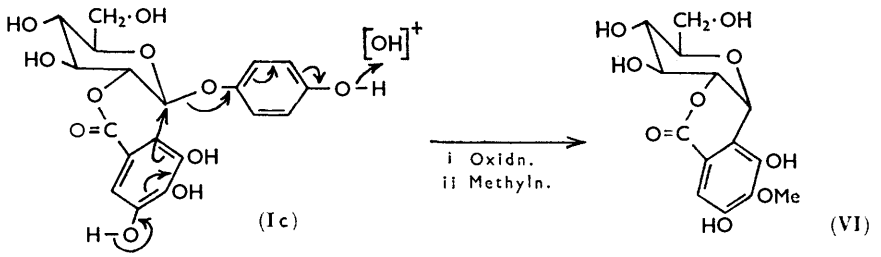


[IVa: $R^1 = R^2 = CO{\cdot}C_6H_2(OH)_3$-3,4,5]
[IVb: $R^1 = H$, $R^2 = CO{\cdot}C_6H_2(OH)_3$-3,4,5]



(V)

be 6-*O*-galloylarbutin (Id) which has been proved by synthesis. Condensation of tri-*O*-benzylgalloyl chloride and *p*-acetoxyphenyl 2,3,4-tri-*O*-acetyl-β-D-glucoside (Ie) [4] followed by hydrogenation and acetylation gave a product identical in all respects with the hepta-acetate of B. Controlled deacetylation gave the natural product. Compound B was also prepared by condensation of equimolar quantities of *p*-benzyloxyphenyl β-D-glucoside (If) [19] and tri-*O*-benzylgalloyl chloride folowed by hydrogenation of the product and presumably, as in related cases,[20] acylation occurs preferentially at the primary alcoholic group of D-glucose. Partial acid hydrolysis of compound C gave mono-*O*-galloylquinol (V) identical with a synthetic product prepared by standard procedures, and the structure of compound C as *p*-galloyloxyphenyl β-D-glucoside (Ig) was corroborated by synthesis. Condensation of *p*-hydroxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (Ih) and tri-*O*-benzylgalloyl chloride followed by hydrogenation and acetylation gave the hepta-acetate of compound C. Controlled deacetylation of the acetate gave the natural product and a further substance provisionally regarded on the basis of previous work [4,21] as its 6-*O*-acetyl derivative (Ii).



(Ic) (VI)

The three *O*-galloyl esters of arbutin (A, B, C; Ic, Id, Ig) were also identified in leaf extracts of *Bergenia crassifolia* and *B. cordifolia*, but in the roots of the same plant species

[19] H. Schiff, *Gazzetta*, 1883, **13**, 538.
[20] O. Th. Schmidt and A. Schach, *Annalen*, 1951, **571**, 29.
[21] Y. Z. Frohwein and J. Liebowitz, *Nature*, 1960, **186**, 153; *Bull. Res. Council Israel*, 1963, **11***A*, 330.

compound A appeared, from a paper-chromatographic analysis, to be replaced by the C-glucoside bergenin [22] (VI). This observation suggested a possible mode of oxidative biosynthesis of bergenin (Ic ⟶ VI) and experimental evidence in favour of these scheme is at present being sought. A further interesting biogenetic relationship was also revealed by a comparison of the phenolic metabolites of *Arctostaphylos uva-ursi* and the closely related member of the same plant family *Vaccinium vitis idaea*.[4] Both plants produce arbutin and derivatives of quercetin (II; R = H) [5,23] but the formation of gallic or the various hydroxycinnamic acid esters appear to be mutually exclusive metabolic functions. Thus only gallic acid derivatives are found in *Arctostaphylos uva-ursi* and cinnamic acid esters in *Vaccinium vitis idaea*. A similar, although less clearly defined, situation exists in the plant family *Aceraceae* [24] where a balance between the two functions appears to operate. The nature of this metabolic relationship between gallic and the hydroxycinnamic acids which is undoubtedly of biogenetic significance remains to be clearly elucidated.

Note added in proof.—The synthesis of bergenin suggests [22] a β-gluosidic structure. The suggested route of biosynthesis indicates the possibility of the alternative α-form (VI).

Experimental

Paper chromatography was carried out by using Whatman No. 1 paper in the solvent systems A, 6% aqueous acetic acid, and B, butan-2-ol–acetic acid–water (14 : 1 : 5) at 20° ± 3°. Arbutin and arbutin derivatives were detected by their blue colorations when sprayed with a 1% methanolic solution of 2,6-dibromobenzoquinone 4-chloroimide (Gibbs reagent) followed by a saturated solution of sodium hydrogen carbonate.[4,25] Galloyl esters were revealed by their absorption or violet fluorescence under u.v. light,[8] by the general *vic*-dihydroxyphenol spray of ferric chloride–potassium ferricyanide (0·2% w/v solutions; 1 : 1) [26] and by a spray of a saturated solution of potassium iodate.[24] Countercurrent distributions were carried out and proton magnetic resonance spectra were measured, as described previously.[4]

Isolation of Phenolic Constituents.—Dried, powdered leaves of *Arctostaphylos uva-ursi* (200 g.; Heath and Heather Ltd., St. Albans) were shaken with water (1000 c.c.) for 24 hr. at 20°, the solution filtered through glass wool, and the pH adjusted to 6·2 by addition of M-potassium dihydrogen phosphate (100 c.c.) and sodium hyroxide solution (2N; approximately 20 c.c.) before extraction with ethyl acetate (10 × 500 c.c.). Removal of the solvent at 30° gave a gum which was freeze-dried from t-butyl alcohol to give a bright yellow amorphous powder (5 g.). Paper chromatography of this extract (solvents A and B) gave the pattern shown in the Table 1. The extract was observed not to contain compounds 28 and 29 when ether was used as solvent instead of ethyl acetate.

The extract (4 g.) was dissolved in acetone (20 c.c.) and separated by thin-layer chromatography (t.l.c.) on cellulose (20 plates; 25 × 25 cm.), 6% aqueous acetic acid being used. Three fractions were taken: I (R_F, 0—0·3, predominantly 1—11); II (R_F, 0·3—0·7, predominantly 12—21); and III (R_F, 0·7—1·0, predominantly 21—29), and the compounds eluted with ethyl acetate (6 × 400 c.c.) and acetone (2 × 250 c.c.). Removal of the solvents gave a gum which in each case was dissolved in water (25 c.c.) and extracted with ethyl acetate (8 × 25 c.c.). Removal of the organic solvent and freeze-drying from t-butyl alcohol gave each fraction as an amorphous powder: I, (1·5 g.); II (1·2 g.); III (0·7 g.).

Fraction I (8 g.) in ethanol at 0° was chromatographed on Nylon (200 g.; 30 × 6 cm.) at 0°. Elution with ethanol (15 × 1000 c.c.) removed flavonoid material, and elution was continued with ethyl methyl ketone–water azeotrope as solvent, fractions (250 c.c.) being collected and analysed by paper chromatography (solvents A, B). Fractions (1—3) contained flavonoids; fractions (4—18) were combined and evaporated to a gum which was freeze-dried from t-butyl

[22] J. E. Hay and L. J. Haynes, *J.*, 1958, 2231.
[23] H. Nakamura, T. Otta, and G. Fukuchi, *J. Pharm. Soc. Japan*, 1935, **55**, 1332.
[24] E. Haslam, *Phytochemistry*, 1965, **4**, 495.
[25] F. E. King, T. J. King, and L. C. Manning, *J.*, 1957, 563.
[26] K. S. Kirby, E. Knowles, and T. White, *J. Soc. Leather Trades' Chemists*, 1953, **37**, 283.

TABLE 1

Compound	R_F A	R_F B	Spray $FeCl_3$–$K_3FeC_6N_6$	Spray KIO_3	Spray Gibbs	u.v.–NH_3	Identification
1	0·03	0·35	—	—	—	Blue	Ellagic acid
2	0·02	0·35	—	—	—	Yellow	Flavonoid
3	0·03	0·60	+	—	—	Yellow-green	Quercetin
4	0·02	0·85	—	—	—	Yellow	Flavonoid
5	0·10	0·20	+	+	—	Absorbs	Ellagitannin (F)
6	0·16	0·32	+	—	—	Yellow	Flavonoid
7	0·18	0·46	+	+	Brown	Absorbs	Gallotannin (D)
8	0·17	0·54	+	—	—	Yellow	Flavonoid
9	0·20	0·72	(+)	—	—	Yellow	Flavonoid
10	0·24	0·90	—	—	—	Yellow	Flavonoid
11	0·26	0·83	—	—	—	Yellow	Flavonoid
12	0·28	0·96	—	—	—	Yellow	Flavonoid
13	0·38	0·20	+	+	Brown	Grey-blue	Corilagin (E)
14	0·45	0·45	—	—	—	Yellow	Flavonoid
15	0·48	0·51	+	+	Blue-brown	Absorbs	Galloylarbutin (C)
16	0·50	0·51	+	+	Blue-brown	Violet	Galloylarbutin (B)
17	0·46	0·68	+	+	Brown	Violet	Gallic acid
18	0·48	0·82	—	—	—	—	Quercetrin
19	0·65	0·44	+	+	Brown	Violet	Galloyl ester
20	0·65	0·62	+	+	Blue-brown	Violet	Galloylarbutin (A)
21	0·68	0·80	—	—	Blue	Blue	Unknown
22	0·76	0·32	+	+	Brown	Violet	Galloyl ester
23	0·76	0·79	+	—	Violet-brown	Absorbs	Unknown
24	0·83	0·90	+	—	Purple	Absorbs	Quinol
25	0·84	0·95	—	—	Blue	Absorbs	Monomethylquinol
26	0·88	0·51	+	—	Blue	Absorbs	Arbutin
27	0·89	0·62	+	—	—	Absorbs	Monomethyl-arbutin
28	0·89	0·68	+		Blue	Absorbs	Pyroside
29	0·90	0·86	+		Blue	Absorbs	Arbutin diacetate

alcohol to give a buff amorphous powder (2·7 g.). This was subject to counter-current distribution (44 transfers) between ethyl acetate and water. Tubes 16—29 were combined and extracted with ethyl acetate (8 × 400 c.c.); removal of the organic solvent and freeze-drying from t-butyl alcohol gave *compound F* as a white amorphous powder (0·112 g.). Tubes 39—45 similarly treated gave *the gallotannin* (*substance D*) (1·79 g.), as a white amorphous powder (Found: C, 52·5, 52·6; H, 4·1, 4·3; glucose 17·5, 17·6. $C_{41}H_{32}O_{26}$ requires: C, 52·3; H, 3·4; glucose 19·2. $C_{48}H_{36}O_{30}$ requires: C, 52·8; H, 3·3; glucose 16·3%. Found: *M*, determined by thermoelectric osmometer in acetone, 980 ± 30. Penta-*O*-galloylglucose requires 940, hexa-*O*-galloylglucose requires 1048). Hydrolysis (N-hydrochloric acid or tannase) [8] of the tannin gave only gallic acid and glucose. Methanolysis [8] of the tannin (0·4 g.) gave β-penta-*O*-galloyl-D-glucose (0·157 g.) (Found: C, 52·5; H, 4·0; glucose 19·1. Calc. for $C_{41}H_{32}O_{26}$: C, 52·3; H, 3·4; glucose, 19·2%), $[\alpha]_D^{20}$ 17·3° (*c* 2 in acetone); R_F (A) 0·075, R_F (B) 0·54. Tetra-*O*-galloyl-D-glucose (0·04 g.) was also isolated [8] from the methanolysis and identified as described previously.[8]

Fraction (III) (3·5 g.) was subjected to counter-current distribution between ethyl acetate and water (50 transfers), and the contents in the tubes were analysed by paper chromatography (solvent B). The contents of tubes 2—8 were crystallised from water, giving arbutin as needles (1·4 g.), m. p. and mixed m. p. with an authentic sample, 200°. Tubes 45—50 similarly gave quinol as needles (0·1 g.), m. p. and mixed m. p. 168—169°.

Fraction II (10 g.) was subjected to counter-current distribution between ethyl acetate and water (50 transfers) and the tube contents were analysed by paper chromatography (solvents A and B). The contents of tubes 5—28 were evaporated to yield a gum (2·4 g.) which was chromatographed in ethanol on Perlon (25 × 2 cm.). Evaporation of the ethanol (500 c.c.) and redistribution of the product between ethyl acetate and water (300 transfers) gave after analysis three fractions composed of tubes 61—100, 161—199, and 14—39. Each, after evaporation, was chromatographed on thin layers of microcrystalline cellulose with 2% aqueous acetic acid as developing solvent, the required band located under u.v. light, and the compound

eluted with aqueous acetone. Finally the compound was redissolved in water (25 c.c.) and re-extracted with ethyl acetate (15 × 25 c.c.). Tubes 61—100 gave on removal of the ethyl acetate a gum, which was dissolved in acetone and treated with benzene giving *compound A* (0·142 g.) as a white amorphous powder (Found: C, 54·1; H, 4·9. $C_{19}H_{20}O_{11}$ requires C, 53·8; H, 4·7%), $[\alpha]_D^{20}$ −7·62° (*c* 3 in acetone–water, 43 : 1); R_F (A) 0·65, R_F (B) 0·62. Compound A crystallised from water as needles, m. p. 164—166° (Found: C, 52·5; H, 5·2. $C_{19}H_{20}O_{11},\frac{1}{2}H_2O$ requires C, 52·6; H, 4·9%); $\nu_{max.}$ (Nujol) 3300, 1685, and 1500 cm.$^{-1}$.

Tubes 161—199, similarly treated, gave *compound B* (0·175 g.) as an amorphous powder (Found: C, 54·0; H, 5·0. $C_{19}H_{20}O_{11}$ requires C, 53·8; H, 4·7%), $[\alpha]_D^{20}$ −31·1° (*c* 3·5 in acetone–water, 43·1); R_F (A) 0·50, R_F (B) 0·51. Compound B crystallised from water as needles, m. p. 227—229° (Found: C, 52·6; H, 4·6. $C_{19}H_{20}O_{11},2H_2O$ requires C, 52·6; H, 4·9%); $\nu_{max.}$ (Nujol) 3300, 1690, and 1500 cm.$^{-1}$.

Tubes 14—39 similarly gave *compound C* (0·132 g.) as an amorphous powder (Found: C, 53·5; H, 4·9. $C_{19}H_{20}O_{11}$ requires C, 53·8; H, 4·7%), $[\alpha]_D^{20}$ −19·7° (*c* 3 in acetone–water, 43 : 1); R_F (A) 0·48, R_F (B) 0·51. Compound C crystallised in needles (from water), m. p. 138—140° (Found: C, 51·2; H, 5·2. $C_{19}H_{20}O_{11},H_2O$ requires C, 51·4; H, 5·0%).

Acid Hydrolysis of Compounds A, B, and C.—The compound (0·01 g.) was heated at 100° with N-hydrochloric acid (1·0 c.c.), and the solution sampled and analysed by paper chromatography after 1 and 12 hr. (see Table 2). After 12 hr. all gave fractions 6, 7, and 11. After 1 hr. compound A gave fractions 1, 6, and 8; compound B fractions 2, 6, and 10, and compound C fractions 3, 10, and 11.

TABLE 2

	R_F		Spray				
Compound	A	B	$FeCl_3$–$K_3FeC_6N_6$	KIO_3	Gibbs	Aniline *	Identification
1	0·65	0·62	+	+	Blue-brown	—	Compound A
2	0·50	0·51	+	+	Blue-brown	—	Compound B
3	0·48	0·51	+	+	Blue-brown	—	Compound C
4	0·76	0·61	+	+	Blue-brown	—	Unknown
5	0·88	0·51	+	—	Blue	—	Arbutin
6	0·83	0·90	+	—	Purple	—	Quinol
7	0·40	0·68	+	+	Purple-brown	—	Gallic acid
8	0·82	0·26	+	+	Purple-brown	+	2-*O*-Galloylglucose
9	0·74	0·24	+	+	Purple-brown	+	6-*O*-Galloylglucose
10	0·20	0·94	+	+	Blue-brown	—	Mono-*O*-galloyl quinol
11	0·98	0·20	—	—	—	+	Glucose

* As aniline hydrogen phthalate.

Action of Base on Compounds A, B, and C.—The compound (0·01 g.) was treated at 20° with 2N-ammonia solution (1·0 c.c.), and the solution analysed by paper chromatography after 2 min., 30 min., and 24 hr. (see Table 2). After 2 min. compound A showed compounds 1, 4, and 2; compound B indicated 2, and compound C, 3. After 30 min. compound A showed compounds 2, 5, and 7; compound B gave 2, 5, and 7, and compound C, 3, 5, and 7. After 24 hr. all showed only fractions 5 and 7.

Action of β-Glucosidase on Compounds A, B, and C.—The compound (0·01 g.) was incubated at 37° for 2 days with the enzyme solution (0·5N-acetate buffer; pH 6·0; 2 c.c.) before paper-chromatographic analysis. Compound C showed the presence of fractions 10 and 11 (see Table 2); compounds A and B were unchanged.

p-*Tri*-O-*benzylgalloyloxyphenyl Acetate.*—A solution of *p*-hydroxyphenyl acetate (3 g.) and tri-*O*-benzylgalloyl chloride (9 g.) in chloroform (50 c.c.) and pyridine (5 c.c.) after 3 days at 20° was diluted with chloroform (100 c.c.), and the organic layer washed successively with water, 2N-hydrochloric acid (2 × 50 c.c.), saturated sodium hydrogen carbonate solution (2 × 50 c.c.), and water. After drying ($MgSO_4$) removal of the chloroform gave a gum which crystallised from alcohol as needles (7 g.), m. p. 138—139° (Found: C, 75·5; H, 5·5. $C_{36}H_{30}O_7$ requires C, 75·3; H, 5·2%); $\nu_{max.}$ (Nujoi) 1755, 1730 cm.$^{-1}$.

p-*Tri*-O-*benzylgalloyloxyphenol.*—The foregoing ester (7 g.) was treated in acetone (300 c.c.) with ammonia solution (s.g. 0·88; 25 c.c.) for 40 hr. at room temperature and the solution poured into ice–water (1000 c.c.) with stirring. After 2 hr. at 0° the precipitate was crystallised

from alcohol to give the *product* as needles (4 g.), m. p. 154—155° (Found: C, 76·8; H, 5·5. $C_{34}H_{28}O_6$ requires C, 76·7; H, 5·3%); $\nu_{max.}$ (Nujol) 3250 and 1710 cm.$^{-1}$.

p-*Galloyloxyphenol*.—*p*-Tri-*O*-benzylgalloyloxyphenol (1·1 g.) was hydrogenated in ethyl acetate (50 c.c.) over a palladium–charcoal catalyst (10%; 0·5 g.) until uptake of hydrogen ceased (24 hr.). Removal of the catalyst and solvent gave a gum which gave p-*galloyloxyphenol* as needles, m. p. 248—250°, from acetone–benzene (Found: C, 59·4; H, 4·2. $C_{13}H_{10}O_6$ requires C, 59·5; H, 3·9%); $\nu_{max.}$ (Nujol) 3300, 1680, and 1600 cm.$^{-1}$; R_F (A) 0·20, R_F (B) 0·92.

p-*Benzyloxyphenyl* D-*Glucoside*.—Arbutin (5·44 g.) dissolved in water (20 c.c.) and ethanol (20 c.c.) was treated with potassium hydroxide (1·12 g.) in aqueous ethanol (50%; 10 c.c.) followed by benzyl chloride (2·53 g.), and the solution heated (steam-bath) for 40 min. After acidification (2N-sulphuric acid) and cooling the product was crystallied (twice) from aqueous ethanol to give p-*benzyloxyphenyl* β-D-*glucoside* as plates (6·3 g.), m. p. 125—126° (Found: C, 63·3; H, 6·2. Calc. for $C_{19}H_{22}O_7$: C, 63·0; H, 6·1%); $\nu_{max.}$ (Nujol) 3300 and 1600 cm.$^{-1}$ (Schiff [19] reports a monohydrate of the above, m. p. 161°, crystallising from water).

p-*Benzyloxyphenyl* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucoside*.—A solution of *p*-benzyloxyphenyl β-D-glucoside (11·5 g.) in acetic anhydride (50 c.c.) and pyridine (50 c.c.), after 24 hr. at 20°, was poured into ice–water, and the precipitate crystallised from ethanol to give the *acetyl derivative* as needles, m. p. 115° (Found: C, 61·1; H, 5·7. $C_{27}H_{30}O_{11}$ requires C, 61·0; H, 5·7%); $\nu_{max.}$ (Nujol) at 1750 and 1500 cm.$^{-1}$.

p-*Hydroxyphenyl* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucoside*.—The above acetate (5 g.) was hydrogenated in ethyl acetate (100 c.c.) over palladium–charcoal (10%; 1·2 g.) for 24 hr. Removal of solvent and catalyst gave p-*hydroxyphenyl* 2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucoside* as plates (4 g.) (from ethanol), m. p. 138° (Found: C, 54·4; H, 5·5. Calc. for $C_{20}H_{24}O_{11}$: C, 54·5; H, 5·5%); $\nu_{max.}$ (Nujol) 3300 and 1750 cm.$^{-1}$.

p-*Tri*-O-*benzylgalloyloxyphenyl* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucoside*.—A solution of *p*-hydroxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (2·15 g.) and tri-*O*-benzylgalloyl chloride (3·25 g.) in chloroform (30 c.c.) containing pyridine (8 c.c.) was maintained at 60° for 7 days, then diluted with chloroform (50 c.c.) and the organic layer washed successively with 2N-hydrochloric acid (2 × 80 c.c.), saturated sodium hydrogen carbonate solution (2 × 80 c.c.), and water. After drying ($MgSO_4$) and removal of the solvent, the residual gum was dissolved in benzene (10 c.c.) and left at 20° for 24 hr. whereafter tri-*O*-benzylgallic anhydride (1·7 g.), m. p. and mixed m. p. 166°, was removed. Addition of light petroleum (b. p. 60—80°) to the filtrate gave needles of the *tri*-O-*benzylgalloyl ester* (2·8 g.), m. p. 167—168° (Found: C, 66·6; H, 5·7. $C_{48}H_{46}O_{15}$ requires C, 66·8; H, 5·4%); $\nu_{max.}$ (Nujol) 1750, 1725, and 1600 cm.$^{-1}$.

p-(3′,4′,5′-*Tri*-O-*acetylbenzoyloxy*)*phenyl* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucoside*.—Hydrogenation of the above ester (2 g.) in ethyl acetate (50 c.c.) over palladium–charcoal (10%, 0·5 g.) was continued for 48 hr., removal of the solvent and catalyst then gave a gum. The latter was treated with pyridine (50 c.c.) and acetic anhydride (20 c.c.) at 20° for 12 hr. before being poured into ice–water. The granular precipitate was crystallised from ethanol to give the acetate as needles (1·5 g.), m. p. 197° (Found: C, 55·5; H, 4·7. Calc. for $C_{33}H_{34}O_{18}$: C, 55·2; H, 4·7%); $\nu_{max.}$ (Nujol) 1780 and 1740 cm.$^{-1}$. The compound was identical (infrared and n.m.r. spectra and m. p.) with the hepta-acetate of compound C.

p-*Acetoxyphenyl* 2,3,4-*Tri*-O-*acetyl*-6-O-(*tri*-O-*benzylgalloyl*)-β-D-*glucoside*.—*p*-Acetoxyphenyl 2,3,4-tri-*O*-acetyl-β-D-glucoside (1·5 g.) and tri-*O*-benzylgalloyl chloride (2·25 g.) were dissolved in chloroform (25 c.c.) containing pyridine (6 c.c.) and the solution maintained at 60° for 7 days, whereafter it was diluted with chloroform and washed successively with 2N-hydrochloric acid (2 × 60 c.c.), saturated sodium hydrogen carbonate solution (2 × 60 c.c.), and water. After drying (Na_2SO_4) and removal of the chloroform the residue was crystallised from ethanol to give the *glucoside* (2·2 g.) as needles, m. p. 164—165° (Found: C, 66·7; H, 5·4. $C_{48}H_{46}O_{15}$ requires C, 66·8; H, 5·4%); $\nu_{max.}$ (Nujol) 1750 and 1730 cm.$^{-1}$.

p-*Acetoxyphenyl* 2,3,4-*Tri*-O-*acetyl*-6-O-(3′,4′,5′ *tri*-O-*acetylbenzoyl*)-β-D-*glucoside*.—The above glucoside (1·2 g.) was hydrogenated in ethyl acetate (35 c.c.) over palladium–charcoal (10%; 0·25 g.) for 24 hr. Removal of the catalyst and solvent gave a gum which was treated directly with acetic anhydride (15 c.c.) in pyridine (30 c.c.) for 12 hr. at 20° and the solution poured into ice–water. Separation of the product and crystallisation from ethanol gave the acetate as needles, m. p. 139—140° (Found: C, 55·2; H, 4·9. Calc. for $C_{33}H_{34}O_{18}$: C, 55·2; H, 4·7%); $\nu_{max.}$ (Nujol) 1775, 1750, and 1730 cm.$^{-1}$. The compound was identical (infrared and n.m.r. spectra and m. p.) with the hepta-acetate of compound B.

p-*Galloyloxyphenyl* β-D-*Glucoside*.—A suspension of *p*-(3′,4′,5′-tri-*O*-acetylbenzoyloxy)-phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (1 g.) in ethanol (20 c.c.) was deoxygenated with hydrogen (2 hr.) and then treated with ammonia (10 hr.) before neutralisation with acetic acid (10 c.c.). Evaporation of the solution gave a gum which was subject to counter-current distribution between ethyl acetate and water (phase volume 40 c.c.; 68 transfers). The tube contents were analysed by paper chromatography, tubes 4—18 combined, and the contents separated further by t.l.c. on cellulose with 2% acetic acid as developing solvent. The major fraction ($R_F \sim 0{\cdot}6$—$0{\cdot}7$) was removed and precipitated from acetone with benzene to give *p*-galloyloxyphenyl β-D-glucoside (0·1 g.) (Found: C, 54·0; H, 5·1. Calc. for $C_{19}H_{20}O_{11}$: C, 53·8; H, 4·7%); $[\alpha]_D^{20}$ −20·2° [*c* 2 in acetone–water (43 : 1)]. The product crystallised from water as needles, m. p. and mixed m. p. with compound C, 136—138° (Found: C, 51·3; H, 5·4. Calc. for $C_{19}H_{20}O_{11},H_2O$: C, 51·4; H, 5·0%); $\nu_{max.}$ (Nujol) 3300 and 1710 cm.$^{-1}$. Acetylation by the usual methods gave the hepta-acetate of compound C.

Tubes (36—40) gave on evaporation and crystallisation *p-galloyloxyphenyl* 6-O-*acetyl*-β-D-*glucoside* as needles, m. p. 181° (Found: C, 51·9; H, 5·3. $C_{21}H_{22}O_{12},H_2O$ requires C, 52·1; H, 5·0%); $[\alpha]_D^{20}$ −32·8° [*c* 1·5 in acetone–water (43 : 1)], $\nu_{max.}$ (Nujol) 3300, 1750, and 1710 cm.$^{-1}$.

p-*Benzyloxyphenyl* 6-O-*Tri*-O-*benzylgalloyl*-β-D-*glucoside*.—A solution of *p*-benzyloxyphenyl β-D-glucoside (1·8 g.) and tri-*O*-benzylgalloyl chloride (2·25 g.) in chloroform (50 c.c.) containing pyridine (6 c.c.) was kept at 45° for 4 days, before dilution with chloroform (50 c.c.). The organic layer was washed with 2N-hydrochloric acid (2 × 100 c.c.), saturated sodium hydrogen carbonate solution (2 × 100 c.c.), and water. After drying (Na_2SO_4) and removal of the solvent the residue was chromatographed over silica gel (150 g.) in ethyl acetate. Fractions (15 c.c.) were collected and analysed by t.l.c. on silica. Fractions (9—15) were combined, concentrated, and further purified by t.l.c. on silica, ethyl acetate being used as developing solvent. The major product (R_F 0·5) was obtained as a gum which was freeze-dried from benzene to give the *ester* (1·5 g.) (Found: C, 59·6; H, 6·0. $C_{47}H_{44}O_{11}$ requires C, 59·9; H, 5·6%).

p-*Hydroxyphenyl* 6-O-*Galloyl*-β-D-*glucoside*.—(*a*) *p*-Benzyloxyphenyl 6-*O*-tri-*O*-benzylgalloyl-β-D-glucoside (1 g.) was dissolved in ethyl acetate (50 c.c.) and hydrogenated over palladium–charcoal (10%; 0·2 g.). Removal of the catalyst and solvent gave a gum which was dissolved in acetone and precipitated with benzene to give *p*-hydroxyphenyl 6-*O*-galloyl-β-D-glucoside as an amorphous powder (0·07 g.) (Found: C, 54·1; H, 4·9. Calc. for $C_{19}H_{20}O_{11}$: C, 53·8; H, 4·7%), $[\alpha]_D^{20}$ −30·9° [*c* 2·5 in acetone–water (43 : 1)]; $\nu_{max.}$ (Nujol) 3300, 1690, and 1610 cm.$^{-1}$. Crystallisation from water gave needles, m. p. and mixed m. p. with compound B, 227—230°.

(*b*) A suspension of *p*-hydroxyphenyl 2,3,4-tri-*O*-acetyl-6-*O*-(3′,4′,5′-tri-*O*-acetylbenzoyl)-β-D-glucoside (1 g.) in ethanol (15 c.c.) was deoxygenated by passage of hydrogen (1½ hr.) after which ammonia was passed (9 hr.). The solution was neutralised by addition of acetic acid (10 c.c.), evaporated to a gum, and subject to counter-current distribution between ethyl acetate and water (phase volume 40 c.c.; 64 transfers). The contents of the tubes were analysed by paper chromatography, and tubes (11—19) combined to give on evaporation a gum. This was dissolved in acetone and *p*-hydroxyphenyl 6-*O*-galloyl-β-D-glucoside precipitated by benzene as an amorphous powder (Found: C, 54·2; H, 4·5. Calc. for $C_{19}H_{20}O_{11}$: C, 53·8; H, 4·7%); $[\alpha]_D^{20}$ −30·6° [*c* 2 in acetone–water (43 : 1)]. Crystallisation from water gave needles, m. p. and mixed m. p. with compound B, 226—228°. Acetylation by the usual methods gave a hepta-acetate identical in all respects with the acetate of compound B.

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