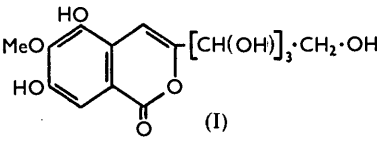
453. *Bergenin, a C-Glycopyranosyl Derivative of 4-O-Methylgallic Acid.*

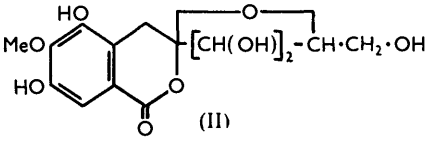
By J. Evelyn Hay and L. J. Haynes.

Bergenin is shown to be the lactone (IV) of 2-β-D-glucopyranosyl-4-*O*-methylgallic acid by studies of light absorption, periodate oxidation, and synthesis.

Bergenin is a colourless crystalline polyphenol which has been isolated from the roots of *Bergenia crassifolia*,[1,2] the bark of *Corylopsis spicata*,[3] and the heartwood of *Shorea leprosula*.[4] There is also evidence [4] that vakerin [5] isolated from the roots of *Caesalpinia digyna* is identical with bergenin. Bergenin was given structure (I) by Tschitschibabin *et al.*[2] in 1928; this was amended to structure (II) by Shimokôriyama [6] in 1950.

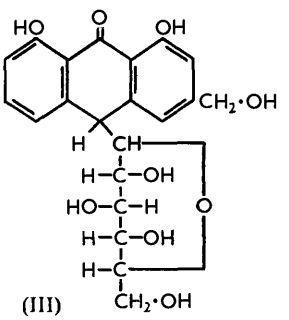


(I)

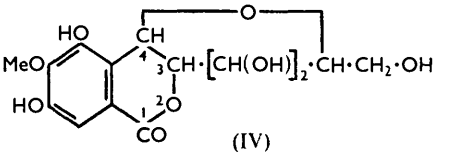


(II)

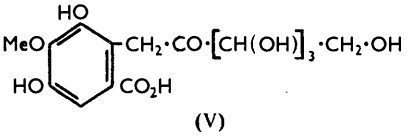
The recent formulation [7] of barbaloin as the *C*-glucopyranosyl compound (III) has led us to search for the presence of this unusual type of sugar linkage in other natural products, and we now present evidence which shows that bergenin should be reformulated as the *C*-glucopyranosyl compound (IV).



(III)



(IV)



(V)

Tschitschibabin *et al.*[2] gave the molecular formula $C_{14}H_{16}O_9,H_2O$ for hydrated bergenin and showed that it contained a lactone ring, one methoxyl group, and two free phenolic hydroxyl groups, but no free carboxyl group. Treatment of bergenin with diazomethane gave a di-*O*-methylbergenin which on permanganate oxidation gave 5 : 6 : 7-trimethoxy-*iso*coumarin-3-carboxylic acid (synthesised by Haworth *et al.*[8] in 1954), 5 : 6 : 7-trimethoxy-*iso*coumarin, and 3 : 4 : 5-trimethoxyphthalic acid. Alkali fusion of bergenin gave 4-*O*-methylgallic acid. Tschitschibabin also reported the formation of a penta-acetyl derivative of bergenin which still contained one active hydrogen atom (Zerewitinoff determination) but could not be further acetylated to yield a hexa-acetate. From these results bergenin was formulated as the *iso*coumarin derivative (I) although no evidence was advanced in support of the linear polyhydroxylic side-chain. Structure (I) does not

[1] Sadikov and Guthner, *Biochem. Z.*, 1927, **190**, 340.
[2] Tschitschibabin, Kirssanow, Korolew, and Woroschzow, *Annalen*, 1929, **469**, 93.
[3] Hattori, *Acta Phytochim., Japan*, 1929, **4**, 327.
[4] Carruthers, Hay, and Haynes, *Chem. and Ind.*, 1957, 76.
[5] Chaudry, Sharma, and Dhar, *J. Sci. Ind. Res., India*, 1954, **13***B*, 147.
[6] Shimokôriyama, *Science, Japan*, 1950, **20**, 576.
[7] Hay and Haynes, *J.*, 1956, 3141; Mühlemann, *Pharm. Acta Helv.*, 1952, **27**, 17.
[8] Haworth, Pindred, and Jefferies, *J.*, 1954, 3617.

explain the resistance to full acetylation and was criticised by Shimokôriyama [6] who proposed the pentahydroxylic structure (II). The evidence for this appears to be based entirely on the fact that bergenin readily reduces Fehling's solution. Shimokôriyama showed that the reducing power of bergenin is comparable with that of glucose, which he explained by formation of a keto-hexose side-chain in ring-opened bergenin (V). It is to be expected from this that di-*O*-methylbergenin would still possess appreciable reducing power. This is not so, and therefore the reducing properties of bergenin itself must be due solely to the presence of the two free phenolic groups and not to a potential reducing group in the side-chain. Moreover, ring-opened di-*O*-methylbergenin does not form a derivative with phenylhydrazine. Since di-*O*-methylbergenin shows no reducing properties, neither structure (I) nor (II) can be correct, since structure (I) should also give the keto-hexose (V) on opening of the lactone ring.

The molecular formula $C_{14}H_{16}O_9,H_2O$ proposed by Tschitschibabin has been confirmed by analysis and by an *X*-ray molecular-weight determination which gave the value 344 ± 4 (required, 346). The carbonyl stretching frequencies in the infrared spectra of bergenin and a number of its derivatives are given in Table 1. It is noteworthy that the

TABLE 1. *Carbonyl stretching frequency of bergenin and its derivatives.*

Compound	ν (cm.$^{-1}$)	Compound	ν (cm.$^{-1}$)
Bergenin hydrate	1699	Tri-*O*-methylbergenin	1737
Anhydrous bergenin	1712, 1682	Penta-*O*-methylbergenin	1740
Di-*O*-methylbergenin	1712	Penta-*O*-acetylbergenin	1780, 1745

TABLE 2. *Ultraviolet-light absorption of bergenin, its derivatives, and related compounds* ($\lambda_{max.}$ *in* mμ).

Compound	$\lambda_{max.}$	log ε	Compound	$\lambda_{max.}$	log ε
Bergenin	275	3·92	*iso*Coumarin	318	3·58
	220	4·42		261	3·87
Di-*O*-methylbergenin	270	3·85		253	3·86
	220	4·42		239	4·22
Penta-*O*-acetylbergenin	254	4·01		228	4·47
	210	4·46	5 : 6 : 7-Trimethoxy*iso*coumarin-	334	3·78
Gallic acid	270	3·89	3-carboxylic acid	298	3·96
	210	4·63		288	3·98
Methyl 4-*O*-methylgallate	275	3·99		252	4·52
	218	4·45	5 : 6 : 7-Trimethoxy*iso*coumarin-	338	3·91
			3-aldehyde	~270	4·12
				247	4·58

position of the lactonic carbonyl stretching frequency in bergenin and its dimethyl ether is lower than would be expected for a δ-lactone, while those for penta-*O*-acetyl- and tri- and penta-*O*-methyl-bergenin are in the usual region of *ca.* 1740 cm.$^{-1}$. Nevertheless, the carbonyl frequency at 1712 cm.$^{-1}$ in the spectra of anhydrous bergenin and its dimethyl ether are in good agreement with the value of 1716 cm.$^{-1}$ quoted by Blair and Newbold [9] for the carbonyl stretching frequency of the lactonic carbonyl group in mellein (3 : 4-dihydro-3-methyl*iso*coumarin), the spectra of both bergenin and mellein being determined on Nujol mulls. The carbonyl stretching frequency in hydrated bergenin appears at 1699 cm.$^{-1}$ and suggests the presence of a free carboxylic acid group; however, bergenin has no acidic properties, and it has been demonstrated chromatographically that the ring-opened form of bergenin is distinct from bergenin itself and only appears after alkaline treatment of bergenin (followed by acidification). Presumably the water of hydration must be associated with the lactone carbonyl group in order to account for this low carbonyl frequency.

The ultraviolet absorption spectra of bergenin and its methyl ethers (see Table 2) are very similar to that of gallic acid and identical with that of methyl 4-*O*-methylgallate, but they bear little resemblance to the spectrum of *iso*coumarin, the broad band at *ca.*

[9] Blair and Newbold, *J.*, 1955, 2871.

320 mμ characteristic of *iso*coumarin and its derivatives being absent. The carboxylic acid and aldehyde derived from di-*O*-methylbergenin by oxidation possess this characteristic *iso*coumarin spectrum, but the absorbing nucleus of bergenin itself must be that of a dihydro*iso*coumarin in which the conjugated system resembles gallic acid. The disappearance of the broad band at *ca.* 320 mμ in the spectra of the dihydro*iso*coumarins is paralleled in the coumarin series, the spectrum of coumarin having a broad band at 330 mμ which is absent in that of dihydrocoumarin.[10]

The position of the methoxyl group in bergenin relative to the two phenolic hydroxyl groups was established by Tschitschibabin who isolated 4-*O*-methylgallic acid on alkali fusion of bergenin: we have confirmed this by the chromatographic identification of 4-*O*-methylgallic acid with one of the products of hydrolysis of bergenin by barium hydroxide, by the identity of the ultraviolet-light absorption of bergenin and methyl 4-*O*-methylgallate, and by the absence of shift in the ultraviolet absorption of bergenin in presence of aluminium chloride.[11]

The substituted dihydro*iso*coumarin nucleus in bergenin accounts for $C_{10}H_8O_5$ of the total $C_{14}H_{16}O_9$ leaving a side-chain (or chains) containing $C_4H_8O_4$ which must be attached to the nucleus by a carbon–carbon linkage through position 3 in order to account for the formation of the trimethoxy*iso*coumarin-3-carboxylic acid on oxidation. Some difficulty was encountered in determining the number of hydroxyl groups present in this side-chain. The original objection to Tschitschibabin's structure for bergenin arose from the formation of a penta-acetate which still contained one active hydrogen atom. We have repeated the preparation of this penta-acetate and found it to contain five acetyl groups and one active hydrogen (Zerewitinoff determination). An *X*-ray molecular weight determination gave the value 530 (required 538). Analysis of the methyl ethers of bergenin however clearly indicated the presence of only five free hydroxyl groups in bergenin, di-, tri-, and penta-*O*-methylbergenin having respectively three methoxyl groups and three active hydrogen atoms, four methoxyl groups and two active hydrogen atoms, and six methoxyl groups and no active hydrogen. The absence of free hydroxyl groups in penta-*O*-methylbergenin was confirmed by an examination of the infrared spectrum. In the spectrum of the penta-acetate the presence of peaks in the region 3000—3600 cm.$^{-1}$ was barely detectable unless an extremely thick Nujol mull was used. In view of the methylation results and of the periodate oxidation studies to be discussed below, it must be concluded that bergenin contains only five free hydroxyl groups and that the active-hydrogen determinations on the penta-acetate are anomalous. It may be noted that a Zerewitinoff determination on glucose penta-acetate has been reported by Lieff, Wright, and Hibbert [12] to give 1·9 and 1·6 active hydrogen atoms in determinations made with dioxan and pyridine solutions respectively.

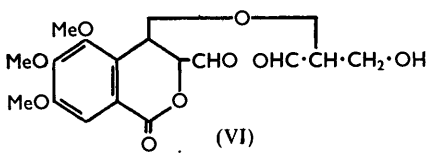
Since bergenin contains only five hydroxyl groups there remains one oxygen atom to be accounted for, and this can only be present as an ether oxygen in the side-chain. Di-*O*-methylbergenin rapidly consumes one mol. of periodate, indicating the presence of only two adjacent hydroxyl groups in the side-chain. When di-*O*-methylbergenin is dissolved in alkali, which opens the lactone ring, and the solution is acidified, it can be shown by paper chromatography that re-formation of the lactone ring is slow and that the hydroxy-acid is stable in dilute acid for about 24 hours. Periodate oxidation of such a ring-opened di-*O*-methylbergenin solution results in a rapid consumption of two mols. of periodate, showing that the two free hydroxyl groups in the side-chain must be adjacent to the hydroxyl group involved in lactone ring formation. A solution of periodate-oxidised di-*O*-methylbergenin began to deposit crystals after several days. Their ultraviolet spectrum (Table 2) indicated the presence of an *iso*coumarin nucleus, and they reduced Fehling's solution and formed a semicarbazone and 2 : 4-dinitrophenylhydrazone and contained an aldehyde

[10] Goodwin and Pollock, *Arch. Biochem. Biophys.*, 1954, **49**, 1.
[11] Swain, *Chem. and Ind.*, 1954, 1480.
[12] Lieff, Wright, and Hibbert, *J. Amer. Chem. Soc.*, 1939, **61**, 865.

group; this group was in conjugation with the *iso*coumarin nucleus since the ultraviolet spectrum showed a bathochromic shift of 20 mμ compared with that of *iso*coumarin and the maxima in the ultraviolet spectra of the derivatives, at 275 mμ for the semicarbazone and 383 mμ for the 2 : 4-dinitrophenylhydrazone, were characteristic of the derivatives of αβ-unsaturated aldehydes. The infrared spectrum of the aldehyde had peaks at 1754 and 1691 cm.$^{-1}$ attributable to the lactonic and the αβ-unsaturated aldehydic carbonyl group respectively. Analyses of the aldehyde and its semicarbazone agreed with the molecular formula $C_{13}H_{12}O_6$, which accommodates the trimethoxy*iso*coumarin nucleus with a substituent aldehydic group. Since the compound is derived from the oxidative breakdown of the side-chain of di-*O*-methylbergenin the aldehydic group must be linked at position 3 of the nucleus and the compound must be 5 : 6 : 7-trimethoxy*iso*coumarin-3-aldehyde.

Reduction of periodate-oxidised di-*O*-methylbergenin with potassium borohydride followed by acidification [13] afforded glycerol, which was detected chromatographically. Periodate oxidation of di-*O*-methylbergenin therefore gives two fragments, the aromatic nucleus with a side-chain of one carbon atom and a chain of three carbon atoms detected ultimately as glycerol, thus accounting for the four carbon atoms in the side-chain of bergenin, which must be linear, and establishing the position of the ether linkage on the third carbon atom of the side-chain. These observations can only be accommodated by structure (IV) for bergenin since structure (II) has been eliminated.

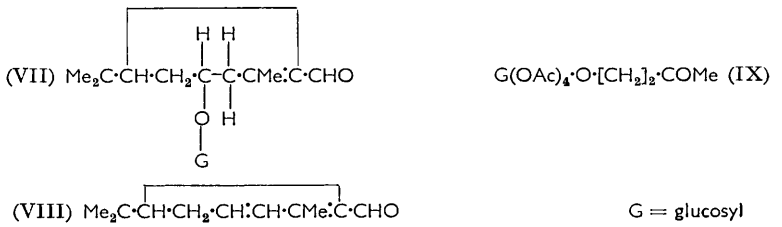
In common with barbaloin (III), bergenin contains the unusual structural feature of a sugar residue attached to the aromatic nucleus by a direct carbon–carbon linkage. Periodate-oxidised barbaloin yields no glycerol on acid treatment of the borohydride reduction product [7] and similar acid-stability of the dialcohol formed by reduction of periodate-oxidised di-*O*-methylbergenin was expected. However it was found that the latter on reduction and treatment with acid yielded glycerol in an amount comparable with that obtained by similar treatment of an *O*-glycosidic compound. The conditions required for cleavage of the ether linkage in periodate-oxidised di-*O*-methylbergenin were therefore studied. Oxidation of di-*O*-methylbergenin by one mol. of periodate must give the dialdehyde (VI) which breaks down slowly at room temperature in the periodate oxidation mixture to give 5 : 6 : 7-trimethoxy*iso*coumarin-3-aldehyde. In the first instance the aldehyde was isolated in low yield from an aqueous solution of di-*O*-methylbergenin which had been oxidised in presence of excess of periodate and set aside for several days before any solid separated. In a controlled oxidation involving equimolar amounts of di-*O*-methylbergenin and sodium metaperiodate, no solid had separated at the end of 16 hours and the ultraviolet absorption of the solution showed maxima characteristic of di-*O*-methylbergenin [the dialdehyde (VI) would however have a similar spectrum]. The solution was neutralised with dilute sodium hydroxide solution, whereupon a flocculent precipitate separated; the solid was isolated and identified by ultraviolet absorption and mixed melting point as the trimethoxy*iso*coumarinaldehyde. The aldehyde also separated from the periodate oxidation mixture on neutralisation with the anion-exchange resin IR-4B(OH^-) and its presence was detected spectrophotometrically by the shift in the ultraviolet absorption of the periodate oxidation mixture after addition of a trace of pyridine, a new maximum at 338 mμ characteristic of the trimethoxy*iso*-coumarinaldehyde appearing in the pyridine-treated solution. It appears therefore that the formation of the trimethoxy*iso*coumarinaldehyde from the dialdehyde (VI) is a base-catalysed reaction analogous to the alkaline hydrolysis of glycosides of alcohols substituted in the β-position by a negative group. Ballou [14] has discussed this group of alkali-sensitive



(VI)

[13] Viscontini, Hoch, and Karrer, *Helv. Chim. Acta*, 1955, **38**, 642.
[14] Ballou, *Adv. Carbohydrate Chem.*, 1954, **9**, 88.

glycosides of which the first known was picrocrocin (VII). Kuhn and Löw [15] observed that picrocrocin breaks down in very dilute alkaline solution to D-glucose and the unsaturated aglucone, safranal (VIII), in one stage for which Isbell [16] has proposed a carbanion mechanism in which the unshared electron pair resulting from extraction of a proton by the base co-ordinates with the adjacent carbon atom with formation of a double bond and simultaneous expulsion of the D-glucosyloxy-anion. The D-glucosides of simpler β-hydroxycarbonyl compounds behave similarly, 3′-oxo-*n*-butyl-β-D-glucopyranoside tetra-acetate (IX) breaking down in dilute alkali to D-glucose and methyl vinyl ketone. Moreover, it has been observed [17,18] that separation of the carbon atom bearing the glycosidic linkage by two or more methylene groups from the negative group results in an alkali-stable glucoside. In support of the proposed mechanism for the breakdown of the dialdehyde (VI) we have found that removal of the carbonyl group by reduction to the dialcohol in buffered borohydride solution [19] resulted in an alkali-stable product which did not yield glycerol on further treatment with alkaline borohydride and acid.



Since cleavage of the ether linkage occurs immediately in alkaline solution it was evident that the isolation of glycerol after borohydride reduction of periodate-oxidised di-*O*-methylbergenin was due, not to acid cleavage of the reductionproduct, but to cleavage of the dialdehyde in the strongly alkaline solution produced on addition of borohydride with subsequent reduction of the glyceraldehyde thus produced. This was substantiated by effecting ether-cleavage in a solution of periodate-oxidised di-*O*-methylbergenin (mole/mole) with dilute sodium hydroxide solution, removal of the trimethoxy*iso*coumarin-aldehyde by extraction into chloroform, and chromatographic detection of glycerol in the aqueous residue after treatment with borohydride.

The structure deduced for bergenin has been finally proved by synthesis. We had no information as to the configuration of the side-chain, but model experiments on the condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with methyl 4-*O*-methylgallate in presence of sodium methoxide, designed to give a compound with a structure similar to that proposed for bergenin, in fact gave bergenin. It seems reasonable to assume that the condensation follows the normal pattern for the formation of glycosides and gives a β-D-glucopyranosyl derivative, and that bergenin is hence the lactone of 2-β-D-glucopyranosyl-4-*O*-methylgallic acid.

Experimental

Bergenin (cf. Tschitschibabin *et al.*[2]).—The roots and rhizomes of *Bergenia crassifolia* (1 kg. wet weight) were thoroughly dried in a vacuum-oven at 60° and ground to powder (446 g.) which was poured into acetone (1 l.) contained in a glass column (24 × 2·5 in.). After 16 hr. the column was eluted with acetone (total volume, 2·5 l.), and three fractions were removed. Paper chromatography of each fraction in butanol–acetic acid–water (4 : 1 : 5) and butanol–ethanol–water (4 : 1 : 5) showed the same phenolic component to be present in each fraction, detected

[15] Kuhn and Löw, *Ber.*, 1941, **74**, 219.
[16] Isbell, *Ann. Rev. Biochem.*, 1943, **12**, 214.
[17] Helferich, Richter, and Flechsig, *Ber.*, 1941, **74**, 1019.
[18] Helferich and Schnorr, *Annalen*, 1941, **547**, 201.
[19] Frush and Isbell, *J. Amer. Chem. Soc.*, 1956, **78**, 2844.

as an orange spot on spraying of the air-dried paper with diazotised sulphanilic acid in 10% aqueous sodium carbonate; no other phenolic products were detected. The fractions were concentrated to brown syrups, dissolved in acetone, and set aside. Crystalline bergenin separated from each fraction, in a total yield of 15 g. Recrystallisation from water yielded the hydrate, m. p. *ca.* 133°. Several recrystallisations from methanol followed by thorough drying (6 hr. at 150°) gave anhydrous bergenin, m. p. 238° [lit., m. p. 130° (hydrate) and 234° (anhyd.)], $[\alpha]_D^{18}$ −37·7° (*c* 1·96 EtOH) (lit., $[\alpha]_D$ −37·3°).

Di-O-methylbergenin (cf. Tschitschibabin *et al.*[2]).—Bergenin (500 mg.) in methanolic solution (10 ml.) was treated with ethereal diazomethane prepared from toluene-*p*-sulphonylmethylnitrosamide.[20] The product (430 mg., 80%) recrystallised from water as a hydrate, m. p. *ca.* 80°; the anhydrous material, m. p. 196° (lit., m. p. 194—196°), was obtained by drying the hydrate over phosphoric anhydride.

Tri- and Penta-O-methylbergenin.—Di-*O*-methylbergenin (3·1 g.) and methyl iodide (50 ml.) were refluxed in dry acetone (85 ml.) until the dimethyl ether had almost completely dissolved. Silver oxide (7 g.) was added in small portions during 3 hr.; dissolution of the dimethyl ether was complete after addition of 2 g. of silver oxide and refluxing for 1 hr. The mixture was refluxed for 1 hr. further, then cooled, the supernatant liquid was decanted and filtered, and the silver residue extracted with hot, dry acetone. The combined filtrate and extracts were concentrated to an orange syrup from which solid separated after storage at 0° overnight. Recrystallisation of the solid from methanol gave needles (300 mg.), m. p. 240—242° [Found: C, 55·3; H, 6·0; OMe, 31·6%; active H, 2·09 atoms. $C_{14}H_{13}O_6(OMe)_3$ requires C, 55·1; H, 5·95; OMe, 33·5%].

The filtrate, after separation of *tri*-O-*methylbergenin*, was concentrated to a red syrup (3·2 g.) which was completely soluble in methanol and methyl iodide. It was dissolved in methyl iodide (50 ml.) and portions of silver oxide (5 g.) were added during 2 hr. with refluxing. After 3 hr. the mixture was cooled, and the orange solution decanted, filtered, and concentrated together with acetone extracts of the silver residues. The orange syrupy residue (3 g.) partially crystallised on treatment with aqueous methanol, giving *penta*-O-*methylbergenin* (600 mg.) which recrystallised from water in needles, m. p. 106° after drying over phosphoric anhydride [Found: C, 57·4; H, 7·0; OMe, 45·3%; active H, 0. $C_{14}H_{11}O_4(OMe)_5$ requires C, 57·3; H, 6·6; OMe, 46·3%].

Penta-O-*acetylbergenin* (cf. Tschitschibabin *et al.*[2]).—Bergenin (100 mg.) was treated with acetic anhydride in dry pyridine solution. The acetate (170 mg.) recrystallised from ethanol as needles, m. p. 207—207·5° (lit., m. p. 199—203°) [Found: C, 53·9; H, 4·7; OAc, 38·0%; active H, 1·01, 1·12; *M*, 530. Calc. for $C_{14}H_{11}O_4(OAc)_5$: C, 53·5; H, 4·9; OAc, 39·9%; *M*, 538].

Periodate Oxidation of Di-O-methylbergenin.—An aqueous solution of di-*O*-methylbergenin (250·9 mg.) and 0·2M-sodium metaperiodate (20 ml.) were mixed, the volume was made up to 100 ml. with distilled water, and the solution set aside at room temperature. A second oxidation mixture containing 277 mg. of di-*O*-methylbergenin was kept at 0°. Within 10 hr. the solution at room temperature had consumed 1·2 mols. of periodate and that at 0° 0·91 mol. of periodate; the periodate uptake was estimated by titration of aliquot parts. The oxidation continued slowly until 2·37 and 2·13 mols. of periodate respectively had been consumed at the end of 5 days.

After 2 days needle-shaped crystals began to separate from the periodate-oxidation mixture, all titrated aliquot portions were retained, and the solids which had separated (60 mg.) were filtered off, washed, dried, and recrystallised by addition of light petroleum (b. p. 60—80°) to a chloroform solution of the solid; it then had m. p. 158—160°.

Periodate Oxidation of Ring-opened Di-O-methylbergenin.—Di-*O*-methylbergenin (136·3 mg.) was dissolved in 0·1N-sodium hydroxide (20 ml.). After 0·5 hr. the pH was adjusted to 5·5 by addition of dilute hydrochloric acid (pH meter). The electrodes were thoroughly rinsed with distilled water, 0·2M-sodium metaperiodate (20 ml.) was added, and the solution made up to 100 ml. A rapid uptake of 2·3 mols. of periodate occurred within 0·5 hr., followed by a slow oxidation rising to 3·4 mols. of periodate consumed during 4 days.

5 : 6 : 7-*Trimethoxy*isocoumarin-3-*aldehyde.*—An aqueous solution (60 ml.) of di-*O*-methylbergenin (500 mg., 0·0014 mole) and sodium metaperiodate (600 mg., 0·0028 mole) was left at room temperature overnight. The acid solution (pH 3·24) was neutralised by dilute sodium

[20] De Boer and Backer, *Rec. Trav. chim.*, 1954, **73**, 229.

hydroxide solution, care being taken to avoid the presence of excess of alkali. The flocculent precipitate, which separated immediately on addition of alkali, was extracted into chloroform. The combined extracts were shaken with dilute aqueous sodium thiosulphate, washed with water, and dried. Removal of the solvent gave a pale yellow *aldehyde* (200 mg., 54%) which, recrystallised from chloroform–light petroleum (b. p. 60—80°), had m. p. 158—160° (Found: C, 59·4; H, 4·6. $C_{13}H_{12}O_6$ requires C, 59·1; H, 4·55%). Its *semicarbazone* formed prisms (from methanol), m. p. 233° (Found: C, 51·8; H, 5·1; N, 13·1. $C_{14}H_{15}O_6N_3$ requires C, 52·3; H, 4·7; N, 13·1%), $\lambda_{max.}$ 360, 275 mμ (log ε 4·26, 4·46).

*Oxidation of Di-*O*-methylbergenin by Periodate* (1 *Mol.*).—An aqueous solution (5 ml.) of di-*O*-methylbergenin (20 mg., 59 mmoles) and sodium metaperiodate (9 mg., 42 mmoles) was left overnight at room temperature. No trimethoxy*iso*coumarinaldehyde was removed by chloroform even after addition of dilute hydrochloric acid. The solution was divided into 3 portions: (*a*) Dilute sodium hydroxide solution was added to neutrality, and a flocculent precipitate separated which was identified as 5 : 6 : 7-trimethoxy*iso*coumarin-3-aldehyde by its ultraviolet absorption ($\lambda_{max.}$ 247, 338 mμ) and by m. p. and mixed m. p. 158—160°. (*b*) Amberlite resin IR-4B(OH^-) was added to neutralise the solution; after 0·5 hr. needle-shaped crystals separated which were identified as the aldehyde. (*c*) One drop of pyridine was added to the solution and the maximum ultraviolet absorption changed from 270 to 338 mμ.

*Reduction of Periodate-oxidised Di-*O*-methylbergenin by Potassium Borohydride* (cf. Viscontini, Hoch, and Karrer [13]).—Sodium metaperiodate (2 mg., 10 mmoles) was added to a solution of di-*O*-methylbergenin (2 mg., 5·9 mmoles) in water (0·2 ml.), and the whole was kept at room temperature for 4 hr. Potassium borohydride (2 mg.) in water (0·1 ml.) was added and the solution kept overnight at room temperature. The solution was heated with N-hydrochloric acid (0·2 ml.) for 15 min. at 100° and placed on a paper chromatogram, with spots of ethylene glycol and glycerol as markers, and allowed to run in ethyl acetate–pyridine–water (10 : 4 : 3). The air-dried paper was sprayed with a mixture of sodium metaperiodate (2%; 4 parts) and alkaline potassium permanganate solution (1% permanganate in 2% aqueous sodium carbonate; 1 part).[21] Glycerol (R_F 0·42) and ethylene glycol (R_F 0·51) yielded yellow spots on a pink ground which developed fully 15 min. after spraying. Di-*O*-methylbergenin gave a yellow spot (R_F 0·42) comparable in intensity with that from sucrose (R_F 0·42) which had been treated in a similar manner.

*Reduction of Periodate-oxidised Di-*O*-methylbergenin by Potassium Borohydride in Buffered Solution.*[19]—Di-*O*-methylbergenin (2 mg.) was oxidised as described above. Amberlite resin IR-120(H^+) was added, followed by potassium borohydride (2 mg.) dissolved in 0·05M-boric acid (0·2 ml.). The acid solution was set aside for 24 hr., made strongly alkaline with aqueous sodium hydroxide, and set aside for a further 24 hr. The solution was then acidified with dilute hydrochloric acid, heated for 15 min. at 100°, and placed on a paper chromatogram. No glycerol was detected by the chromatographic method described above.

Reduction of the Periodate Oxidation Mixture after Alkali Cleavage and Removal of 5 : 6 : 7-*Trimethoxy*iso*coumarin-3-aldehyde.*—The aldehyde was removed by chloroform extraction of a solution of periodate-oxidised di-*O*-methylbergenin which had been neutralised with dilute aqueous sodium hydroxide, and potassium borohydride was added to the aqueous residue. After 16 hr. the solution was acidified with dilute hydrochloric acid, heated at 100° for 15 min., and placed on a paper chromatogram. Glycerol was detected by the method described above.

Hydrolysis of Bergenin by Barium Hydroxide.—Bergenin (20 mg.) in saturated barium hydroxide solution (3 ml.) was heated at 100° for 2 hr. in a sealed tube. The orange solution was acidified by passing it through a column of Amberlite resin IR-120(H^+), and concentrated *in vacuo* to a yellow syrup which was placed on a paper chromatogram and allowed to run in butanol–acetic acid–water (4 : 1 : 5) and butanol–ethanol–water (4 : 1 : 5). Spraying the air-dried papers with a solution of diazotised sulphanilic acid in aqueous sodium carbonate revealed three phenolic components, of R_F 0·56 (yellow), 0·65 (orange), and 0·75 (red), in the neutral solvent; none of these components was identical with bergenin (R_F 0·48). The spots of R_F 0·56 and 0·65 were shown to be acidic by spraying a second paper with an aqueous solution of potassium iodate, potassium iodide, and starch (equal proportions of 0·5% iodate solution and 1% iodide solution containing 0·4% of starch), the acidic components of the mixture giving blue spots on a white ground. The faster-moving acid component ran at the same rate in the

[21] Lemieux and Baur, *Analyt. Chem.*, 1954, **26**, 920.

two solvent systems, and gave the same colour reaction with diazotised sulphanilic acid, as 4-*O*-methylgallic acid (prepared by hydrolysis of methyl 4-*O*-methylgallate [22]).

Ring-opening of Bergenin and Di-O-methylbergenin.—Bergenin and di-*O*-methylbergenin were dissolved in dilute sodium hydroxide solution, and the solutions acidified with dilute hydrochloric acid and placed immediately on a paper chromatogram along with bergenin and di-*O*-methylbergenin as markers. The mixture was allowed to run in ethyl acetate–acetic acid–water (3 : 1 : 3), the paper was dried in air, and the free acids were detected by spraying with a solution of potassium iodide, potassium iodate, and starch, followed by overspraying with an alkaline solution of diazotised sulphanilic acid. Bergenin had R_F 0·59, the dimethyl ether R_F 0·76, and the free acids R_F 0·46 and 0·65 respectively. The free acid represented *ca.* 90% of the mixture immediately after acidification and ring closure occurred slowly at room temperature, *ca.* 40% of free acid remaining after 24 hr.; however after the solutions had been heated at 100° for 0·5 hr. only a trace of the free acids was detected chromatographically.

Synthetic Bergenin.—The course of the reaction was followed chromatographically, the solvent system being ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4) and the spray reagent an alkaline solution of diazotised sulphanilic acid.

Sodium methoxide in methanol (4·8 g. of sodium in 150 ml.) was added to a methanolic solution (200 ml.) of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (20 g.) and methyl 4-*O*-methylgallate (10 g.) (prepared by Schöpf and Winterhalder's method [22]). The red solution was left *in vacuo* overnight, diluted with water (200 ml.), and made weakly acid by addition of 2N-acetic acid (85 ml.) Methanol was removed from the clear orange solution by distillation *in vacuo* and the aqueous residue extracted with ether. Methyl 4-*O*-methylgallate (6·5 g.) was recovered from this extract after drying and removal of solvent. The aqueous residue was shown chromatographically to be free from methyl 4-*O*-methylgallate, but contained three phenolic components (R_F 0·43, 0·50, and 0·60). The component of R_F 0·43 was unaffected by hot mineral acid, travelled at the same rate, and gave the same colour reaction with the spray reagent, as bergenin. The components of R_F 0·50 and 0·60 were separated by development on 3 MM paper and elution of the appropriate bands with boiling ethyl acetate. Both components were sensitive to hot mineral acid, the former (R_F 0·50) yielding glucose, methyl 4-*O*-methylgallate, and 4-*O*-methylgallic acid and the latter (R_F 0·60) giving bergenin; hence the first must be the *O*-glycoside of methyl 4-*O*-methylgallate and the second product the methyl ester of the hydroxy-acid formed on ring-opening of bergenin.

Accordingly the aqueous residue from ether-extraction was made strongly acid with 5N-hydrochloric acid and heated for 2 hr. on the water-bath to destroy the acid-labile products. The yellow solution was cooled, and extracted with ether to remove methyl 4-*O*-methylgallate and 4-*O*-methylgallic acid formed on hydrolysis of the *O*-glycoside, and the residue extracted repeatedly with ethyl acetate. The combined extracts were dried and the solvent was removed leaving an orange syrup (1·3 g.) which was dissolved in a small volume of hot water and set aside. Bergenin separated as the hydrate (250 mg.), m. p. and mixed m. p. with the natural product, 130—133°. Several recrystallisations from ethyl acetate followed by thorough drying gave anhydrous bergenin, m. p. and mixed m. p. 230°, $[\alpha]_D^{18}$ −39·3° (*c* 1·908 in MeOH) (lit.,[4] $[\alpha]_D$ −38·5° in MeOH). This gave a penta-acetate, needles (from ethanol), m. p. and mixed m. p. 208°. The infrared spectra of synthetic and natural bergenin and of the penta-acetyl derivatives were identical.

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[22] Schöpf and Winterhalder, *Annalen*, 1940, **544**, 62.