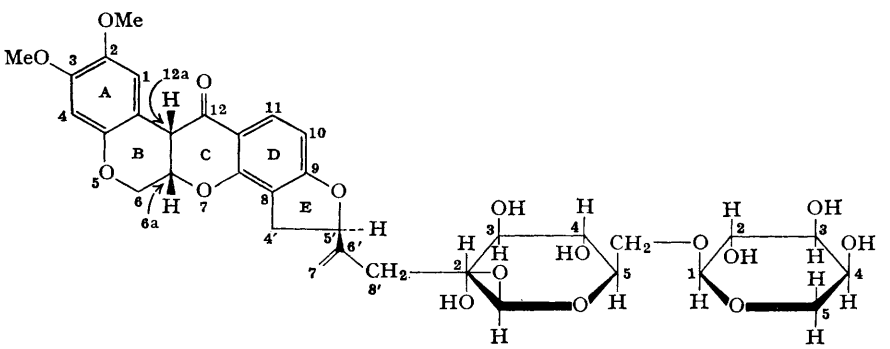
1162. *Structure and Stereochemistry of the Vicianoside Amorphin, the First Rotenoid Glycoside.*

By J. Claisse, L. Crombie, and R. Peace.

On acidic or enzymic hydrolysis amorphin yields glucose, arabinose, and amorphigenin. The presence of fragment (III) in the latter is demonstrated by spectroscopic means, by degradation to various oxidation levels of the B/C system, and by other standard rotenoid reactions. The remaining five carbon atoms and the primary hydroxyl group are also placed by spectroscopic and chemical means. Stereochemical features at 6a, 12a, and 5′ are determined and the suggested structure and stereochemistry (IV) for amorphigenin is checked by hydrogenolysis to products identical with those from the degradation of rotenone.

The disaccharide fragment from amorphin is isolated by hydrogenolysis and shown to be 6-*O*-α-L-arabinosyl-D-glucose (vicianose) by chemical degradation. Amorphin, the first glycosidic rotenoid, is as shown in (I).

Amorpha fruticosa (Leguminosae: division Papilionaceae), a native of N. America which has been naturalised in Europe and W. Asia, has been the subject of various chemical investigations. The leaves are said to contain apigenin glucoside[1,2] and the seeds yield an insecticidal or insect-repellent extract.[3,4] From this extract a glycoside amorphin, together with small amounts of its aglycone amorphigenin, were isolated by Acree and his colleagues.[5,6] Acid



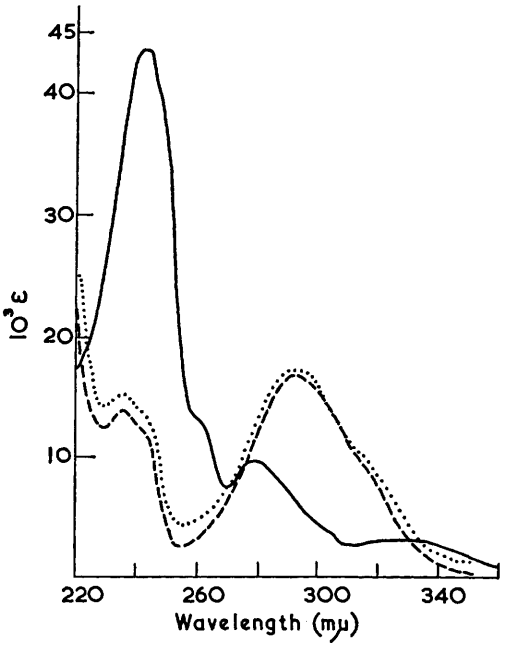
(I)

hydrolysis of amorphin yielded amorphigenin,[6] the rotenoid character of which was suspected from its positive Durham test. This has been supported by recent Russian work[7–11] which is mentioned later. Our investigation has led to the complete structure and stereochemistry (I) for amorphin.

Amorphin reduced Fehling's solution only weakly and, as this slow reduction almost certainly involves the aglycone, amorphin is classed as a "non-reducing" glycoside. On

1 Goto and Taki, *J. Pharm. Soc. Japan*, 1938, **58**, 933.
2 Zemplen and Mester, *Ber.*, 1943, **76**, 776.
3 Brett, *J. Agric. Res.*, 1946, **73** (3), 81.
4 Brett and Hodnett, *J. Econ. Entomol.*, 1947, **40**, 593.
5 Acree, Jacobson, and Haller, *Science*, 1944, **99**, 99.
6 Acree, Jacobson, and Haller, *J. Org. Chem.*, 1943, **8**, 572.
7 Kondratenko and Abubakirov, *Doklady Akad. Nauk. S.S.S.R.*, 1962, **146**, 1340.
8 Kondratenko and Abubakirov, *Uzbek. Khim. Zhur.*, 1962, **6**, 73.
9 Kondratenko and Abubakirov, *Uzbek. Khim. Zhur.*, 1962, **6**, 60.
10 Kondratenko and Abubakirov, *Uzbek. Khim. Zhur.*, 1961, **5**, 66.
11 Kondratenko and Abubakirov, *Doklady Akad. Nauk Uzbek. S.S.S.R.*, 1960, **35**.

hydrolysis with 5% sulphuric acid or Amberlite IR-120 two sugars and amorphigenin* were produced. The structure of the latter is considered first. Acree and his colleagues[6] considered amorphigenin to have molecular formula $C_{22}H_{22}O_7$, but Kondratenko and Abubakirov use $C_{22}H_{20}O_7$.[7,10] The molecular formula used by us, $C_{23}H_{22}O_7$, is based on a consistent pattern of analyses for derivatives of amorphigenin, n.m.r. proton counts on the latter, and on amorphigenin itself, and on a mass spectral molecular weight determined on amorphigenin. Combustion analyses on amorphigenin itself conflicted with those of its derivatives and this was shown to be due to tenacious crystal-solvation by methanol, benzene, and acetone. The foreign absorptions (τ 6·53, 2·66, 7·85 in the three cases mentioned) were readily observed in the n.m.r. spectra and close examination of infrared spectra revealed the expected minor disturbances. An *X*-ray molecular weight determination conflicted with the mass spectral determination for the same reason.



Ultraviolet spectra of isorotenone (——), amorphin (·····), and amorphigenin(– – – –).

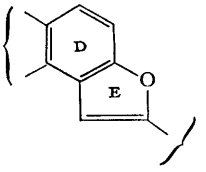
A striking feature of the preliminary examination was similarity of the ultraviolet spectra of amorphin, amorphigenin, and rotenone. The infrared spectrum of amorphigenin was also similar to that of rotenone except that the former had a strong hydroxyl absorption. It had a band at 1669 cm.$^{-1}$ in the usual position for an unbonded rotenoid 12-carbonyl (rotenone 1674 cm.$^{-1}$).[12] Amorphigenin contained two methoxyl groups (n.m.r. and Zeisel) and one olefinic double bond as judged by microhydrogenation. The latter could not be in an isorotenone-type[13] situation [cf. (II)] because the ultraviolet spectrum of isorotenone is different from that of amorphigenin (Figure). Our initial effort was to establish the presence of fragment (III) and to show that the hydroxyl was not located in this part of the molecule.

Nuclear magnetic resonance assignments for amorphigenin are given in structure (IV). The characteristic ring D *ortho*-hydrogen quartet, τ 2·16 and 3·51 (J = 9 c./sec.), present in

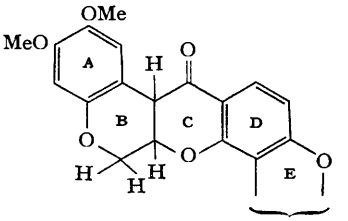
* For a preliminary communication on the constitution of amorphigenin see Crombie and Peace, *Proc. Chem. Soc.*, 1963, 246.

[12] Büchi, Crombie, Godin, Kaltenbronn, Siddalingaiah, and Whiting, *J.*, 1961, 2843.
[13] Crombie, *Fortschr. Chem. org. Naturstoffe*, 1963, **21**, 111.

rotenone at τ 2·19 and 3·54 (J = 8 c./sec.)[14] shows the angular C D E arrangement. The *para*-hydrogens of ring A in amorphigenin (J very small) are represented by singlets at τ 3·23 and 3·56 (rotenone τ 3·24 and 3·58). As in rotenone (τ 6·18 and 6·21) the two methoxyls are not identical (τ 6·18 and 6·22). Comparison of the multiplet near τ 5·5 in rotenone with that in amorphigenin shows, apart from an intrusive band in the latter at τ 5·73, considerable similarity.

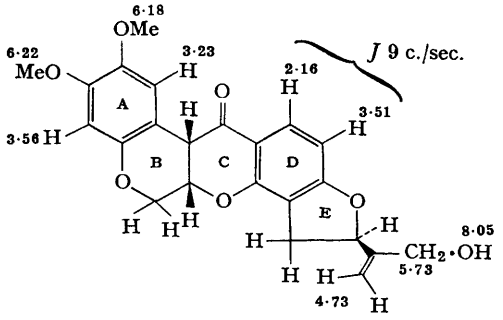


(II)

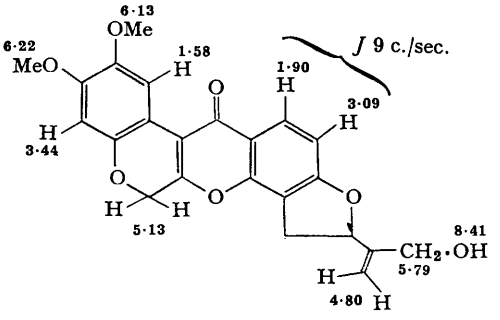


(III)

Chemical evidence supports the presence of fragment (III) still more clearly. When amorphigenin is treated with iodine and sodium acetate in ethanol, 6a,12a-dehydroamorphigenin (V) is formed. The hydroxyl group is still present (3460 cm.$^{-1}$, mull; cf. acetate formation) so it is not at 6a or 12a, and the 12-keto-peak is characteristically shifted to 1634 cm.$^{-1}$ (dehydrorotenone 1634 cm.$^{-1}$).[15] 6a,12a-Dehydroamorphigenin is optically active, so like rotenone but unlike deguelin there is dissymmetry elsewhere than at 6a and 12a. Nuclear magnetic resonance assignments are shown on diagram (V) and are as expected from our earlier work.[14] The 1-hydrogen is now more efficiently negatively shielded (τ 1·58) by the 12-carbonyl as it is more nearly in plane and the 10,11-doublet is at rather lower τ values. The multiplet due to the 6,6a,12a-hydrogens of amorphigenin has contracted to a sharp band at τ 5·13 due to the 6-methylene. Dehydroamorphigenin and dehydrorotenone have very similar ultraviolet spectra.



(IV)

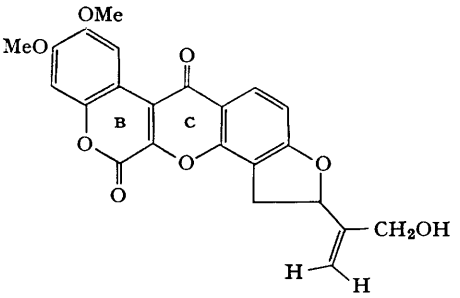


(V)

When treated with nitrous acid 6a,12a-dehydroamorphigenin was oxidised to the keto-lactone (VI), $\nu_{max.}$ (mull) 3448 (hydroxyl), $\nu_{max.}$ ($CHCl_3$) 1733 (lactone) and 1645 (12-ketone) cm.$^{-1}$ [cf. rotenonone 1730 and 1645 cm.$^{-1}$].[14] Ultraviolet credentials of the keto-lactone are like those of rotenonone. This establishes chemically that the hydroxyl could not be at 6, 6a, or 12a and the n.m.r. evidence shows its absence from all other positions in (III). The formation of a rotenonone-type also proves that amorphigenin is truly a rotenoid and not an isoflavanone. Keto-lactone (VI) was too insoluble for n.m.r. study but its optically active acetate was examined. The 6-methylene band in (V) had disappeared, the acetate methyl was at 7·97, and the 1- and 4-hydrogens were at τ 1·05 and 3·14. Compared with dehydroamorphigenin acetate, the 1- and 4-hydrogens had fallen appreciably in τ value (from 1·58

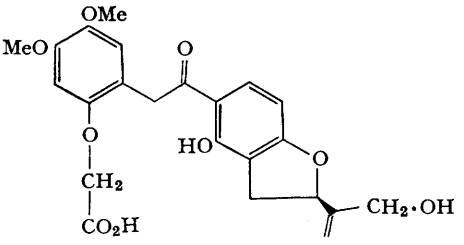
[14] Crombie and Lown, *J.*, 1962, 775.
[15] Crombie and Whiting, *J.*, 1963, 1569.

and 3·46). This may be due to the introduction of pyrone resonance in ring B as well as C although long-range shielding by the 6-carbonyl system is an alternative possibility. Pyrone resonance in ring B as well as C would lead to juxtaposition of two positively charged rings which might be mutually destabilising but there is no evidence for this from the τ values of the 10,11-protons which are in much the same position (3·02 and 1·83) as in dehydroamorphigenin (3·09 and 1·90). The acetate of compound (V) analyses as expected for $C_{25}H_{20}O_9$ and the proton integral supports this, but the parent compound (V) analyses as a monohydrate.

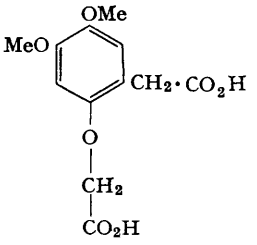


(VI)

Hydrolysis of dehydroamorphigenin with 5% ethanolic potassium hydroxide gave the optically active derrisic acid relative (VII), spectroscopically similar to derrisic acid[13] and oxidised by alkaline hydrogen peroxide to derric acid (VIII). When refluxed with acetic anhydride and anhydrous sodium acetate the acid (VII) re-formed the acetate of dehydroamorphigenin. The 12-keto-group in amorphigenin is reduced by sodium borohydride[12] to

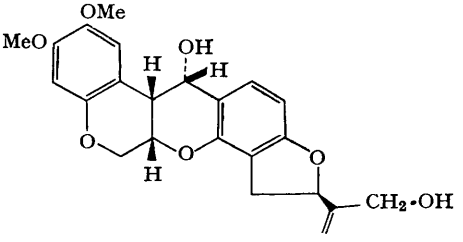


(VII)

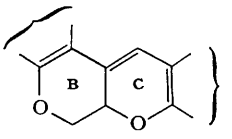


(VIII)

give the diol (IX), with drastic alteration of the ultraviolet spectrum. The diol can be dehydrated to the stilbene-like system (X) (identified spectroscopically). The above information establishes the presence of fragment (III) in amorphigenin and there now remains the nature and placing of the hydroxyl function and the five carbon atoms attached at C-8.



(IX)

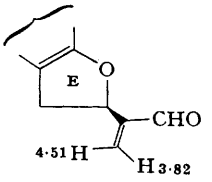


(X)

The hydroxyl group showed an infrared maximum at 3498 cm.$^{-1}$ under high resolution ($c < 0{\cdot}005$M in CCl_4) and must be internally hydrogen-bonded to oxygen rather than an aromatic or olefinic system. That the peak at τ 8·05 represents the hydroxyl proton in amorphigenin was shown by its disappearance on deuteration and by its behaviour with acid. The peak at τ 5·73 appeared to represent the methylene of a primary hydroxyl, not noticeably spin-split, and this was confirmed by the characteristic downfield shift to τ 5·43 on acetylation. The properties of amorphigenin acetate require explanation. It was first encountered during attempts to induce an "isorotenone"-type change[13] in ring E of amorphigenin by treatment with concentrated sulphuric acid–acetic acid, and had m. p. 166—167°, $[\alpha]_D^{19\cdot5} - 106{\cdot}6°$ (c 1·2 in $CHCl_3$), and ultraviolet and infrared spectral evidence, 1669 cm.$^{-1}$ (12-carbonyl), 1736 cm.$^{-1}$ (acetate), left no doubt that it was not a 12-enol acetate. Treatment of amorphigenin with isopropenyl acetate–sulphuric acid gave the same product, but hot acetic anhydride–sodium acetate gave an impure product, m. p. 144—147°, $[\alpha]_D^{20\cdot2} - 88{\cdot}8°$, and only after a number of crystallisations were the m. p. and rotation raised to 157° and $[\alpha]_D^{21\cdot6} - 106{\cdot}4°$. It seems that the acetic anhydride–sodium acetate racemises the 6a,12a-system as happens in the case of rotenone[13] [when amorphigenin is refluxed with ethanolic sodium acetate the rotation falls from $-125{\cdot}6°$ ($CHCl_3$) to $-68{\cdot}2°$]. As one B/C fusion is thermodynamically much more stable than the other, this results in the appearance of a pair of diastereoisomers, the 5′-centre remaining unracemised. Consequently a number of crystallisations are needed to isolate one diastereoisomer. Acid conditions do not racemise the 6a,12a-system (or the 5′) and this is so in the case of rotenone. Hence these conditions give one diastereoisomer directly.

When amorphigenin was shaken with manganese dioxide in chloroform at 20° 6a,12a-dehydrogenation[16] did not occur, but a new compound with carbonyl bands at 1681 and 1689 cm.$^{-1}$ was formed. The bands assigned to the CH_2·OH group had disappeared in the n.m.r. spectrum but a new single proton characteristic for an aldehyde (τ 0·33) had appeared. This represents oxidation of an allylic or benzylic primary alcohol to an aldehyde, and from this, and the information above, it is clear that C:C·CH_2·OH is present.

A complete structure (IV) can now be written for amorphigenin. The two 4′-protons are represented in the n.m.r. by a multiplet near τ 6·7 and the 5′-proton by a multiplet near 4·6. The 7′-vinyl group is represented by the unresolved two-proton band near τ 4·73. In rotenone and dehydrorotenone the vinyl group is a doublet (each member probably being split again); the two vinyl protons in these cases are in rather different chemical environments but in amorphigenin and its dehydro-compound the environments are more similar and the two signals are degenerate. When the acetate of amorphigenin or its dehydro-compound is made, the long-range shielding of the acetyl causes the two 7′-hydrogens to have different chemical shifts and a doublet appears again. In the case of the aldehyde (XI) the vinyl proton *cis* to the aldehyde group falls to τ 3·82 (cf. methyl angelate and tiglate[17]).

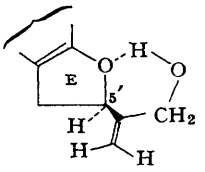


(XI)

The *cis*-B/C fusion of amorphigenin follows from the τ value 3·23 for the 1-hydrogen; it has been shown elsewhere that the *cis*-series have values near 3·3 and the *trans* near 2·0.[14,18] Rotenone and all other rotenoids so far examined have positive Cotton effects and, so far

[16] Crombie, Godin, Whiting, and Siddalingaiah, *J.*, 1961, 2876.
[17] Jackman and Wiley, *J.*, 1960, 2886.
[18] Crombie and Lown, *Proc. Chem. Soc.*, 1961, 299.

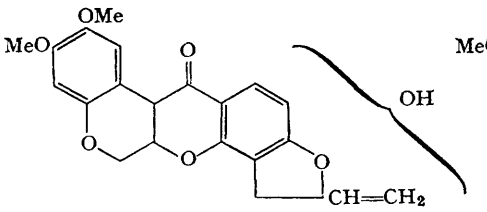
as the 6a,12a centres are concerned, form one stereochemical series.[15,19] Amorphigenin is no exception. Finally, the configuration at 5′ has been compared with that in rotenone.[12] For this purpose the dissymmetry at 6a and 12a had to be destroyed and the o.r.d. curve of the acid (VII) was compared with that of derrisic acid.[19,20] Both showed similar negative plain curves. The stereochemistry of amorphigenin is thus 6aβ,12aβ,5′β, as in compound (IV). The hydroxyl stretching position, at 3498 cm.$^{-1}$, is accounted for as the hydroxyl is well oriented to form a six-membered hydrogen-bonding ring (XII). Other evidence for the



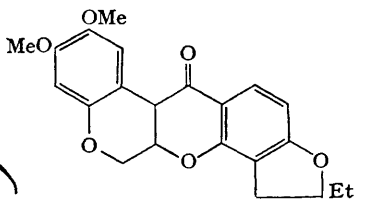
(XII)

presence of this feature is the lowered position of the 5′-proton in the n.m.r. spectrum (τ 4·6) relative to that in rotenone (τ 5·1—5·2) and other models like lunacrine and columbianetin.[21] The more fixed vinyl group causes greater deshielding of this proton. In solution, physical evidence suggests that the more bent *cis*-conformer formula (XII) in ref. 12 is preferred (n.m.r., hydrogen bonding evidence in rotenolones) but the unit cell in amorphigenin will not accommodate this. The second and rather more extended *cis*-conformer [formula (XI) in ref. 12] must give a more compact crystal-lattice.

As confirmation of structure (IV), amorphigenin was hydrogenated over Adams platinum in acetic acid. Chromatography gave 6′,7′-dihydrorotenone formed by hydrogenolysis of the 8′-allylic hydroxyl and 6′,7′-hydrogenation, and 12-deoxy-6′,7′-dihydrorotenone formed by further reduction of the 12-ketone followed by hydrogenolysis of the benzylic hydroxyl. These were identical with specimens derived from rotenone[12] and effect a convincing structural and stereochemical link between the two natural products. 12-Deoxy-6′,7′-dihydroamorphigenin was also isolated from the hydrogenolysis products. During our work a series of papers on amorphigenin by Kondratenko and Abubakirov appeared. These authors worked to the formula $C_{22}H_{23}O_7$, and their researches culminated[7] in a suggestion of formula (XIII). They consider[7,9] that a product obtained by hydrogenation and hydro-



(XIII)



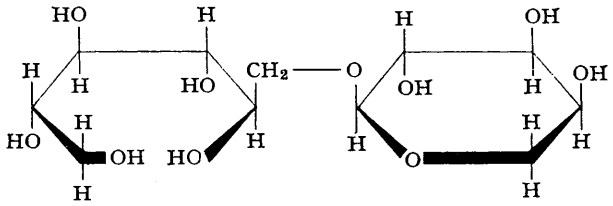
(XIV)

genolysis of amorphin is (XIV) by comparison with a synthetic product described in the literature.[22] Their product should be stereochemically homogeneous at 6a, 12a, and 5′ whereas that in the literature[22] must be one (±)-diastereoisomer or a mixture of (±)-diastereoisomers. Particularly as no mixed m. p.s were carried out we consider that the comparison is unconvincing even though it was extended to 6a,12a-dehydro-compounds [the natural is optically active at 5′, the synthetic (±) at 5′]. It is suggested that Kondratenko and Abubakirov's product may be impure 6′,7′-dihydrorotenone.

[19] Djerassi, Ollis, and Russell, *J.*, 1961, 1448.
[20] Crombie and Peace, *J.*, 1961, 5445.
[21] Varian Catalogue of N.M.R. Spectra (Vol. 1), Varian Associates, Palo Alto, California, 1962.
[22] Miyano and Matsui, *Chem. Ber.*, 1960, **93**, 54.

Using the methods of Jermyn and Isherwood[23] it was shown, in agreement with the report of Kondratenko and Abubakirov,[11] that two sugars, glucose and arabinose, were produced on acid hydrolysis of amorphin. These were separated by thin-layer chromatography and their proportions found to be 1 : 1 by the dichromate oxidation technique of Jaynne and Hahn.[24] Amorphin was unaffected by α-glucosidase but hydrolysed by β-glucosidase.[25] The product was not a disaccharide but D-glucose plus L-arabinose and this is presumably because the β-glucosidase preparation contains α- and β-galactosidases the latter of which would split off the homomorphic L-arabinose α-linked as its pyranoside.[26] Partial acid hydrolysis of amorphin gave mixtures of aglycone, unchanged glycoside, and the two monosaccharides.

In order to isolate the disaccharide, amorphin itself was subjected to hydrogenolysis with Adams catalyst in acetic acid. This gave dihydrorotenone together with dihydrodeoxyrotenone and the disaccharide which was purified by preparative paper-sheet chromatography. Colour reaction (*p*-anisidine–acid) indicated that the reducing end of the disaccharide was the aldohexose rather than the aldopentose.[27] This arrangement, which agrees with Russian views,[10] was confirmed by reduction with sodium borohydride to give the alditol (XV) which on acid hydrolysis gave arabinose and a non-reducing component identified chromatographically by comparison with the alcohol formed by borohydride reduction of glucose.



(XV)

The aglycone amorphigenin was found in test experiments to react with nearly one mol. of periodate to give the 6a,12a-dehydro-compound. Correcting for this, the glycosidic portion of amorphin destroyed 4 mol. of periodate under standard conditions, no formaldehyde being formed. This means that the arabinose must be linked to position 2 or 6 of the glucose residue. That the linkage is to position 6 was shown by complete methylation of amorphin by treatment with barium hydroxide and methyl iodide in dry dimethyl sulphoxide,[28] followed by methanolysis and gas-chromatographic identification of the methylated sugars on butanediol succinate polyester and polyphenyl ether columns.[29] The methyl glucosides of 2,3,4-trimethylarabinose and 2,3,4-trimethylglucose (α and β) were produced. On acid hydrolysis of the disaccharide, the rotation increased indicating an α-L-arabinose linkage.[26]

These results show that the disaccharide is 6-*O*-α-L-arabinosyl-D-glucose and that it is β-linked to amorphigenin. Thus the complete structure for amorphin is as shown in (I). The disaccharide, vicianose,[30] has been found in nature before. It is said to occur as a glycoside in *Vicia sativa* (vicianin),[31] *Geum urbanum* (gein),[32] and *Viola* sp. (violutoside)[33] and a synthesis has been reported.[34]

[23] Jermyn and Isherwood, *Biochem. J.*, 1949, **44**, 402.
[24] Jaynne and Hahn, *Angew. Chem.*, 1960, **72**, 520.
[25] Bailey and Pridham, *Adv. Carbohydrate Chem.*, 1962, **17**, 121.
[26] Pigman, "The Carbohydrates," Academic Press, New York, 1957.
[27] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[28] Srivastava, Harshe, and Singh, *Tetrahedron Letters*, 1963, 1869.
[29] Aspinall, *J.*, 1963, 1676.
[30] Wallenfels and Beck, *Annalen*, 1960, **630**, 46.
[31] Bertrand and Weisweiller, *Compt. rend.*, 1910, **150**, 180; 1910, **151**, 325, 884.
[32] Herissey and Cheymol, *Compt. rend.*, 1925, **180**, 384; **181**, 565.
[33] Picard, *Compt. rend.*, 1926, **182**, 1167.
[34] Helferich and Bredereck, *Annalen*, 1928, **465**, 166.

Experimental

Except where otherwise stated, the following apply. Ultraviolet spectra were determined for ethanol solutions and infrared spectra for chloroform solutions with sodium chloride optics. Evaporation signifies evaporation under reduced pressure and drying refers to the use of anhydrous sodium or magnesium sulphate. In chromatographic work the letters N, A, or K together with a numeral refer to neutral, acid, or alkaline alumina of the numbered Brockmann grade.

Isolation of Amorphin.—Seeds of *A. fruticosa* (2·56 kg.) were coarsely ground and extracted in a Soxhlet apparatus with light petroleum (b. p. 40—60°). Evaporation of the extract gave a brown waxy product (254 g.). The petrol-extracted seeds were extracted with methylene chloride to give a black tarry product (54 g.). Finally, extraction was continued with methanol. On concentration and keeping at 0°, crude glycoside (40·5 g.) crystallised and was washed with methanol, acetone, or methylene chloride. Thin-layer chromatography (Merck Kieselgel G, eluant methanol, iodine spray) gave three yellow spots R_F 0·12, 0·73, and 0·82. Quantitative reproducibility of such R_F figures is not high. Attempted crystallisations from pure methanol gave gums, but addition of a few drops of water caused separation of fine white needles. Purified amorphin had m. p. 154—155° (to a glass) and gave only one spot on thin-layer chromatography as above (R_F 0·69). The glycoside gave a positive Durham test (indigo)[35] but the Rogers and Calamari test[36] was negative possibly because of insolubility; it reduced Fehling's solution slowly. Amorphin had $[\alpha]_D^{18\cdot5}$ −123·6° (*c* 1·1 in methanol), $[\alpha]_D^{24\cdot5}$ −87·9° (*c* 2·55 in pyridine), $[\alpha]_D^{22\cdot4}$ −69·6° (*c* 1·7 in dimethylformamide); $\lambda_{max.}$ 236 (15,400), 240s (14,100), and 293 (17,140) mμ; $\nu_{max.}$ (mull) 3436 (strong, hydroxyl), 1675 (ketone), and 1608 (aryl) cm.$^{-1}$. Lit.,[6,11] m. p. 151—151·5°, $[\alpha]_D^{20}$ −67·9° ± 0·5° (*c* 4·044 in pyridine).

Combustion analyses of amorphin by four microanalysts have produced discordant results, frequently on the same sample sealed *in vacuo*. This appears to be a consequence of solvation and hygroscopic character. The molecular formula accepted, $C_{34}H_{40}O_{26}$, and used for calculation of the physical data, is derived from degradation and identification of the fragments. Dr. M. Elliott (Rothamstead Experimental Station) reports that amorphin is almost non-toxic towards mustard beetles.

Isolation of Amorphigenin.—Crude amorphin (29 g.) was refluxed with 5% sulphuric acid (3·63 l.) for 3½ hr. The solid dissolved giving a clear solution which then became cloudy and deposited crude amorphigenin (19 g.) as a white precipitate. This was washed with water and dried at 100°. Crude amorphigenin (10 g.) was chromatographed (50 × 6·5 cm. column) from chloroform, using benzene–chloroform (1:1) as eluant, on deactivated alumina (3 lb.). The latter was prepared by shaking Spence "Type H, 100—200-mesh" activated alumina (3 lb.) with 10% acetic acid (68 ml.) for 10 min. and allowing it to cool to room temperature. On crystallisation from chloroform–ethanol fractions 15—18 gave amorphigenin as needles, m. p. 195—196·5°. Small quantities of 6a,12a-dehydroamorphigenin, m. p. 228°, presumably formed by aerial oxidation, were also isolated in some experiments.

Pure amorphigenin had m. p. 196—197° when crystallised from benzene–chloroform or ethanol–chloroform (lit.,[6,11] 191—192°); it could also be crystallised from acetone–water or n-butanol. The aglycone gave no ferric reaction but showed positive Durham and Rogers and Calamari tests. Solvation caused analytical complications. Material was crystallised from methanol–chloroform (methanol detected by n.m.r. spectrum, τ 6·53) (Found: C, 64·5, 64·65, 65·25, 65·25, 64·95, 65·3; H, 5·65, 6·00, 5·8, 5·55, 5·85, 5·75. Calc. for $C_{23}H_{22}O_7.CH_3{\cdot}OH$: C, 65·15; H, 5·9%. Calc. for $C_{23}H_{22}O_7$: C, 67·3; H, 5·4%).

A specimen crystallised from benzene was orthorhombic, *c* being the axis of the needle. Unit-cell dimensions were: $a = 15{\cdot}883 \pm 0{\cdot}03$; $b = 28{\cdot}933 \pm 0{\cdot}07$; $c = 13{\cdot}687 \pm 0{\cdot}02$ Å. The density of the crystals (flotation) was $1{\cdot}349 \pm 0{\cdot}01$ giving 5108 ± 80 as the weight of cell contents. Other considerations suggest that there are 12 molecules in this cell (made up of three pseudo-cells, each containing four molecules) giving a molecular weight of $425{\cdot}7 \pm 7$. The dimensions of the pseudo-cells, 15·9 × 28·9 × 4·6 Å, suggest an extended molecule with the length of the molecule lying close to the direction of the *b*-axis.

Examination by mass-spectral means showed a parent molecular ion at $m/e = 410$ with a fairly abundant parent minus two ion at $m/e = 408$ (probably 6a,12a-dehydroamorphigenin). Ions at 395 and 382 correspond to loss of methyl and a CO molecule respectively. The base peak is at m/e 192.

[35] Jones and Smith, *Ind. Eng. Chem., Analyt.*, 1933, **5**, 75.
[36] Rogers and Calamari, *Ind. Eng. Chem., Analyt.*, 1936, **8**, 135.

Amorphigenin had $[\alpha]_D^{20}$ −125·6° (*c* 2·04 in $CHCl_3$); $\lambda_{max.}$ 236 (13,900), 242s (12,300), and 293 (16,900) mμ; $\nu_{max.}$ 1669 (12-carbonyl), 1605 (aryl), and $\nu_{max.}$3498 (hydroxyl; *c* < 0·005M in CCl_4, grating). In the n.m.r. spectrum the following assignments were made: τ 2·16 (doublet, *J* = 9 c./sec., 11-H), 3·51 (doublet, *J* = 9 c./sec., 10-H), 3·23 (singlet, 1-H), 3·56 (singlet, 4-H), 4·73 (not resolved, two vinyl protons), 5·73 (two protons α to hydroxyl), 6·18 and 6·22 (two OMe groups), 8·05 (hydroxyl—disappears on shaking with D_2O), near 4·6 (multiplet, 5′-H), 5·0—6·2 (multiplet, 6a, 12a, and two 6-H), near 6·7 (multiplet, 4′-H).

Apart from detection of solvation by n.m.r. methods, careful study of infrared mull spectra also reveals this. Thus, the methanol solvates have an extra band at 1025 $cm.^{-1}$ (cf. liquid methanol 1030 $cm.^{-1}$) which disappears on crystallisation from benzene and is replaced by intensification of bands at 688 and 677 $cm.^{-1}$ and appearance of a new one at 1038 $cm.^{-1}$ [cf. liquid benzene 1037 and 679 (broad) $cm.^{-1}$].

The optical rotatory dispersion curves of rotenone and amorphigenin were measured in dioxan with the following result (molecular rotations in parentheses). Amorphigenin methanol solvate: 357·5 mμ (+4210° first extremum), 348·5 (0°), 343 (−2005°, second extremum, inflexion), 331·5 (−6210° third extremum), 317 mμ (0°). Rotenone: 358 (+3080° first extremum), 351·5 (0°), 344 (−2400° second extremum), 329 mμ (0°).

Hydrolysis of Amorphin with Ion-exchange Resin.—Amberlite IR-120 resin was washed on a sinter with three times its "wet volume" of 0·5N-sulphuric acid, then with water until the washings were neutral, and finally with ethanol. It was dried at 30—40°. Amorphin (110 mg.) in distilled water (25 ml.) was stirred under reflux with IR-120 resin (550 mg.) for 6 hr., progress of the reaction being followed by thin-layer chromatography. Extraction with ether gave amorphigenin (18 mg.), m. p. and mixed m. p. 183—184° (and infrared comparison).

The aqueous layer was filtered and evaporated to give a gum (62 mg.). By chromatography on Whatman No. 1 paper (28 cm. square) using in one case the top layer from n-butanol–ethanol–water (4:1:5 v/v), and in another the non-aqueous layer from ethyl acetate–water–acetic acid (3:3:1 v/v), the gum was shown to contain glucose and arabinose. Development was in each case continued for 24 hr., the lower edge of the paper being cut to a point to allow solvent to drain away evenly. In the first eluant the gum gave two spots (distance moved 9·70 and 8·00 cm.) in agreement with these being arabinose (9·65 cm.) and glucose (7·90 cm.). In the second eluant the gum gave two spots (distance moved 7·05 and 12·30 cm.) again in agreement with identification as glucose (7·00 cm.) and arabinose (12·15 cm.). The developer was ammoniacal silver nitrate followed by heating at 100° for 30 min. Kondratenko and Abubakirov[11] have reported the isolation of L-arabinose and D-glucose (the latter as the osazone).

Hydrolysis of Amorphin with β-Glucosidase.—Amorphin (0·9 g.) was suspended in McIlvaine citrate buffer (pH 5·0) (180 ml.) containing β-glucosidase (0·9 g., Seravac Ltd.) through which a slow stream of nitrogen pre-saturated with water vapour was passed. The mixture was kept at 37° and the experiment continued for 20 days. Extraction with chloroform and evaporation gave amorphigenin (144 mg.), m. p. and mixed m. p. 187—189° after crystallisation from benzene–chloroform. Identity was confirmed by infrared and n.m.r. spectra. Material insoluble in both the buffer and chloroform (362 mg.) was shown to be impure unchanged amorphin.

In a similar experiment unattacked amorphin was filtered off and amorphigenin was extracted with chloroform. The residual aqueous solution was passed through ion exchange columns of IR-120 (H) and IR-4B resins, evaporated *in vacuo*, and then chromatographed on thin-layer plates using kieselguhr buffered with sodium acetate (0·02M) and eluting with ethyl acetate–n-butanol–water (57:32:13) (chromogenic spray: *p*-anisaldehyde–sulphuric acid). Heating at 100° gave spots of the correct R_F and colour for D-glucose and L-arabinose (see below). No other reducing sugar spots could be detected.

Treatment of Amorphin with α-Glucosidase.—Amorphin (0·87 g.) and maltase (1 g.) in citrate buffer (180 ml.) at pH 5·0 were kept at 35·2° for one month in a stream of nitrogen saturated with water. Filtration gave unchanged impure amorphin (0·805 g.). Extraction with chloroform gave no amorphigenin. The aqueous layer was treated as in the β-glucosidase experiment but no spots corresponding to glucose, arabinose, or any oligosaccharides could be detected. The only spot was one of high R_F, identified as being due to the original glycoside.

Identification and Estimation of the Constituent Monosaccharides by Thin-layer Chromatography.—The filtrate from the acid hydrolysis of amorphin was neutralised by passage through a column of IR-4B ion-exchange resin and was then evaporated to dryness and treated with charcoal in hot ethanol. Evaporation gave a syrup, $[\alpha]_D^{28·5}$ +79° ± 8° (*c* 0·7 in water).

Thin-layer chromatography from acetone on kieselguhr buffered with sodium acetate (0·02M) followed by drying and spraying with *p*-anisaldehyde (0·5 ml.)–sulphuric acid (0·5 ml.) in ethanol (9 ml.) and heating at 80° for 10 min. revealed the sugars. Two spots R_F 0·70 (yellow-green) and 0·48 (blue) were formed. Arabinose gave a yellow-green spot, R_F 0·71, and glucose a blue spot, R_F 0·50.

The hydrolysate sugars in methanol were spotted along the starting line (0·25 cm. intervals) of a thin-layer plate and chromatographed as above. Elution was continued until the solvent front reached the 10 cm. line and the plate was dried and sprayed as above. A second plate, treated identically, was prepared at the same time and with the sprayed plate as a guide the thin-layer adsorbent areas containing each of the two sugars were removed. Each adsorbent area was heated with standardised potassium dichromate (0·05M) in 70% sulphuric acid (7 ml.) at 95—100° for 1 hr. The solution was cooled, diluted with water (20 ml.), and 5% potassium iodide solution (5 ml.) was added. Liberated iodine was back-titrated with 0·01N-sodium thiosulphate after 2 min., starch indicator being used. Blank runs were carried out and a correction was made for the dichromate used. Duplicate determinations gave a glucose:arabinose ratio of 1:0·95 and 1:1·01.

Oxidation of Amorphin with Periodate.—Amorphin (10 mg.) was accurately weighed and dissolved in 50% aqueous ethanol (40 ml.). Freshly prepared sodium metaperiodate (0·3M) (2 ml.) was added and made up exactly to 50 ml. At intervals samples (2 ml.) were withdrawn, made up to 50 ml. with phosphate buffer (pH 7), and samples (10 ml.) were withdrawn and excess of 20% potassium iodate was added. The liberated iodine was titrated with 0·01N-sodium thiosulphate, starch indicator being used.

Other samples (2 ml.) were withdrawn for formaldehyde estimation and mixed with freshly prepared and filtered barium chloride–sodium hydrogen carbonate solution (2 ml.) and kept for 10 min. The solution was centrifuged and samples (2 ml.) were withdrawn and mixed with phenylhydrazine reagent (2 ml.) and kept in the dark for 30 min. Phenylhydrazine reagent was made by heating phenylhydrazine hydrochloride in water (1:3 w/v) with a little charcoal for 30 min., filtering hot, and pouring the filtrate into concentrated hydrochloric acid (1:3 v/v). When the mixture had cooled, the solid was filtered off, washed with acetone, dried *in vacuo* (P_2O_5), and dissolved in 0·2N-sodium acetate buffer (pH 3·5) to give a 1% solution. A 2% solution of recrystallised potassium ferrocyanide in concentrated hydrochloric acid (7 ml.; 2:5 v/v) was added and the solution was kept for 3 min. and made up to 50 ml. The purple solution was shaken well and its optical absorption at 518 mμ compared with that from a blank control run. Solutions of amorphigenin and glucose were treated similarly with the following results:

	Mol. of IO_4^- consumed	U.v. absorption (*E*)
Amorphin	4·7 ± 0·2	0·007
Amorphigenin	0·8 ± 0·2	0·011
Glucose	5·1	0·234

Hydrogenolysis of Amorphin.—Amorphin (5 g.) was hydrogenated over pre-reduced Adams platinum catalyst (0·5 g.) in acetic acid (200 ml.) until 2 mols. of hydrogen had been absorbed. The product was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was extracted first with chloroform and then with water. On chromatography on deactivated alumina from benzene, the chloroform extract (0·51 g.) gave dihydrorotenone and dihydro-12-deoxyrotenone, m. p. 215° and 171° (and mixed m. p.), respectively. The identification was confirmed by infrared spectral comparison.

Thin-layer chromatography of the aqueous extract on sodium acetate buffered (0·02M) kieselguhr using ethyl acetate–n-butanol–water eluant (57:32:13), with the *p*-anisidine chromogenic reagent, gave a brown spot, R_F 0·48, due to the amorphin oligosaccharide (vicianose). On the same plate arabinose (pink-brown) had R_F 0·74 and glucose (brown), R_F 0·65.

Isolation of the Oligosaccharide from Amorphin by Paper Chromatography.—Whatman No. 3 paper (30·5 × 28·5 cm.) was cut to a point at one of the longer edges to aid drainage and spots of crude amorphin oligosaccharide (from hydrogenolysis) were put at intervals (2·5 mm.) along a starting line drawn on the long side opposite to the point. After equilibration (12 hr.), downward elution was carried out for 30 hr., the upper layer of an ethyl acetate–water–acetic acid (3:3:1) mixture being used. The paper was air-dried and strips cut from the sides and centre of the paper; the sugar was located with a silver nitrate–ammonia spray and heat (100°). With these as guides, the main area of paper containing the oligosaccharide was cut away and the

sugar was isolated by extraction with methanol. Evaporation gave a gum, $[\alpha]_D^{24}$ +41° final (*c* 0·46 in water); it was kept in aqueous methanol at low temperature. Lit.[34] for vicianose, slow mutarotation from $[\alpha]_D^{14}$ +56·6° → +40·5° final (water).

Reduction of the Oligosaccharide with Sodium Borohydride.—A few drops of an aqueous solution of amorphin oligosaccharide were reduced with a few drop of aqueous sodium borohydride solution (100 mg./ml.) at 20° for 20 min. The solution was acidified with drops of sulphuric acid (3N) until no more hydrogen was evolved; two further drops of acid were added and the solution was heated on a steam-bath for 3 hr. and then cooled. Barium carbonate was added until effervescence ceased and the supernatant solution was chromatographed on kieselguhr buffered with sodium acetate (0·02M) on a thin-layer plate, elution being effected with acetone. The spray reagent was *p*-anisaldehyde–sulphuric acid. It was found that aldoses were not satisfactorily separated from alditols under these conditions but aldoses gave much brighter-coloured spots. Arabinose had R_F 0·68 (yellow-green), arabitol 0·69 (dull red), glucose 0·51 (blue), sorbitol 0·51 (dull red). The hydrolysed amorphin oligosaccharide reduction product gave spots with R_F 0·68 (yellow-green) and 0·51 (dull red), indicating arabinose and sorbitol.

Methylation of Amorphin.—Amorphin (1 g.) was refluxed and continuously stirred with methanol (6 ml.) and methyl iodide (1·5 ml.) while dry silver oxide (3 g.) was being added. The mixture was heated for a further 2 hr., filtered, and evaporated to dryness *in vacuo*. The residue was heated to 70° with water (2 ml.). Dimethyl sulphate (4 ml.) and 40% sodium hydroxide (5 ml.) were added dropwise during 20 min., the solution being kept just alkaline throughout the addition. The reaction mixture was then heated to 100° for 30 min. and cooled. The solution was neutralised with sulphuric acid, and the product was extracted with chloroform. Evaporation of the extract gave a yellow-brown methylation product.

The methylation product was methanolysed and subjected to gas chromatography according to Aspinall's method.[29] By means of a butane-1,4-diol succinate polyester column at 175°, the main components detected were methyl glycosides of 2,3,4-trimethylarabinose (*T* 1·04), 2,3,4-trimethylglucose (β) (2·59) and 2,3,4-trimethylglucose (α) (3·70). Lit.,[29] *T* = 1·04, 2·59 and 3·70, respectively. Lesser amounts of various dimethylarabinoses were also found. The time *T* is the retention time relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as standard. By means of a polyphenyl ether column at 200°, the main components detected were again methyl glucosides of 2,3,4-trimethylarabinose (0·83), 2,3,4-trimethylglucose (β) (1·35), and 2,3,4-trimethylglucose (α) (1·82). Lit.,[29] *T* = 0·83, 1·35, and 1·83. Methylation by the technique of Srivastava *et al.*[28] gave an almost completely methylated product and the dimethylarabinose contaminants were eliminated from the gas-chromatographic traces.

Amorphigenin Acetate.—(*a*) Amorphigenin (250 mg.) was stirred in glacial acetic acid (13 ml.) while concentrated sulphuric acid (10 ml.) was added dropwise and the temperature kept below 40°. After the cherry red solution had stood for 30 min. it was poured into iced water and extracted with ether. The ethereal extract was washed with sodium hydrogen carbonate and water, and dried and evaporated to give crude acetate (111 mg.), m. p. 159·5° after crystallisation from ethanol–chloroform. The *acetate* was chromatographed from benzene on alumina (A3) and then crystallised from ethanol; it formed needles, m. p. 166—167° (Found: C, 66·2, 66·55, 66·6; H, 5·55, 5·3, 5·35. $C_{25}H_{24}O_8$ requires C, 66·35; H, 5·35%), $[\alpha]_D^{19·5}$ −106·6° (*c* 1·2 in $CHCl_3$); $\lambda_{max.}$ 235 (14,900), 241s (13,300), and 292 (17,900) mμ; $\nu_{max.}$ ($CHCl_3$) 1736 (acetate), 1669 (12-ketone), and 1608 (aromatic) cm.$^{-1}$. The n.m.r. resonances were: τ 2·16 (doublet, *J* = 9 c./sec., 11-H), 3·48 (doublet, *J* = 9 c./sec., 10-H), 3·22 (singlet, 1-H), 3·52 (singlet, 4-H), 4·78 and 4·86 (vinyl protons on C-7′, lower-field proton assigned to that on the same side of the acetate), 5·43 (two protons α to acetate), 6·28, 6·32 (two OMe groups), 8·00 (acetate methyl protons), near 4·6 (multiplet, 5′-H), 5·1—6·2 (multiplet, 6-protons), near 6·8 (multiplet, 4′-protons), and 5·6—6·2 (multiplet, 6a,12a-protons).

(*b*) Amorphigenin (250 mg.) was refluxed for 30 min. with isopropenyl acetate (10 ml.) containing 5 or 6 drops of concentrated sulphuric acid. The product was poured into water and worked up as above to give amorphigenin acetate, m. p. and mixed m. p. with the above specimen 166—166·5° (and infrared spectral comparison).

(*c*) Amorphigenin (300 mg.) and anhydrous sodium acetate (150 mg.) were heated at 100° with acetic anhydride (12 ml.) for 1 hr. and poured into water. The crude acetate (303 mg.), m. p. 144—147°, $[\alpha]_D^{20·2}$ −88·8° (*c* 2·4 in $CHCl_3$), was recrystallised successively from methanol with the following result: (i) m. p. 150—154°, softened 144°; (ii) 154°, softened 144°; (iii) 157°, softened 154°. A mixed m. p. with the specimen m. p. 166—167° above gave no depression and

infrared chloroform and ultraviolet spectra were identical. Only after several more recrystallisations did the rotation reach $[\alpha]_D^{21.5}$ −106·4° (*c* 1·3 in $CHCl_3$).

Acid Hydrolysis of Amorphigenin Acetate.—The acetate (75 mg.) was refluxed with 10% aqueous sulphuric acid (10 ml.) in dioxan (5 ml.) for 75 min. Cooling, extraction with chloroform, washing, drying, and evaporation gave amorphigenin (35 mg.), m. p. 188° undepressed by authentic material, and having an identical mull infrared spectrum.

Racemisation of Amorphigenin at Positions 6a *and* 12a.—Amorphigenin (250 mg.) was refluxed for 2 hr. with sodium acetate (500 mg.) in ethanol (50 ml.). The product was poured into water, extracted with chloroform, and the extract when evaporated gave a gum, $[\alpha]_D^{18.5}$ −68·2° (*c* 2·98 in $CHCl_3$).

6a,12a-*Dehydroamorphigenin.*—Amorphigenin (2 g.) and freshly fused sodium acetate (5 g.) were dissolved in refluxing ethanol (50 ml.), and iodine (1·6 g.) in ethanol (13 ml.) was added dropwise during 90 min. Refluxing was continued for a further hour and the product was kept overnight at 0°. Filtration gave 6a,12a-dehydroamorphigenin (1·18 g.), m. p. 224—225° (decomp.). Chromatography on alumina (A3) from benzene–chloroform (1:1) followed by crystallisation from chloroform–ethanol gave the *dehydro-compound* as pale yellow needles, m. p. 228·5—229·5° (decomp.) (Found: C, 67·6, 67·35, 67·2; H, 4·94, 5·05, 4·9. $C_{23}H_{20}O_7$ requires C, 67·65; H, 4·95%), $[\alpha]_D^{19}$ −50·8° (*c* 0·83 in $CHCl_3$); $\lambda_{max.}$ 238 (28,700), 279 (23,400), and 309 (17,900) mμ; $\nu_{max.}$ ($CHCl_3$) 1634 (12-carbonyl) and 1608 (aryl) cm.$^{-1}$ with hydroxyl absorption at 3460 cm.$^{-1}$ in the mull spectrum. There were n.m.r. resonances at τ 1·58 (1-H), 1·90 (doublet, J = 9 c./sec., 11-H), 3·09 (doublet, J = 9 c./sec., 10-H), 3·44 (4-H), 4·80 (7′-vinyl), 5·13 (6-methylene), 5·79 (8′-protons), 6·13, 6·22 (two methoxyls), 8·41 (8′-hydroxyl), near 4·5 (multiplet, 5′-H), near 6·6 (multiplet, 4′-protons).

6a,12a-Dehydroamorphigenin (100 mg.), anhydrous sodium acetate (50 mg.), and acetic anhydride (4 ml.) were heated on steam for 1 hr. and poured into iced water. The crude product (81 mg.), m. p. 177·5°, was chromatographed on alumina (A3) in benzene and crystallised from chloroform–ethanol to give 6a,12a-*dehydroamorphigenin acetate*, m. p. 179° (Found: C, 66·15, 66·8; H, 5·1, 5·05. $C_{25}H_{22}O_8$ requires C, 66·65; H, 4·9%), $[\alpha]_D^{22}$ −40·7° (*c* 0·92 in $CHCl_3$); $\lambda_{max.}$ 238 (29,600), 280 (23,900), and 309 (17,700) mμ; $\nu_{max.}$ ($CHCl_3$) 1733 (acetate), 1637 (12-ketone), and 1608 (aromatic) cm.$^{-1}$. There was no hydroxyl absorption in the mull infrared spectrum. There were n.m.r. resonances at τ 1·58 (1-H), 1·91 (doublet, J 9 c./sec., 11-H), 3·11 (doublet, J 9 c./sec., 10-H), 3·46 (4-H), 4·73 and 4·80 (7′-vinyl protons), 5·13 (6-protons), 5·38 (protons α to acetate), 6·12, 6·22 (two OMe groups), 7·98 (acetate methyl), near 4·5 (multiplet, 5′-H), near 6·5 (multiplet, 4′-protons).

The Keto-lactone (VI).—Dehydroamorphigenin (500 mg.) was dissolved in warm glacial acetic acid (10 ml.) and cooled in ice. Pentyl nitrite (1·75 ml.) was added before the compound had completely crystallised followed by a mixture of concentrated hydrochloric acid (1 ml.) and glacial acetic acid (1 ml.) added dropwise with agitation. The mixture was kept at 0° (1 hr.), allowed to warm to 20°, and then filtered. The *keto-lactone* (VI) (434 mg.) was washed with ethanol and then crystallised from chloroform–ethanol as yellow needles, m. p. 300° (decomp.) (Found: C, 62·75, 62·75, 62·55, 62·95, 62·35; H, 4·7, 4·6, 4·7, 4·55, 4·25. $C_{23}H_{18}O_8,H_2O$ requires C, 62·7; H, 4·6%. $C_{23}H_{18}O_8$ requires C, 65·4; H, 4·3%); $\lambda_{max.}$ ($CHCl_3$) 261 (23,600), 268 (23,150), 298 (19,700), and 340 (8200) mμ; $\nu_{max.}$ ($CHCl_3$) 1733 (lactone) and 1645 (12-carbonyl) with a hydroxyl band at 3448 cm.$^{-1}$ in the infrared mull spectrum.

The keto-lactone (100 mg.) was refluxed in acetic anhydride (4 ml.) with anhydrous sodium acetate (50 mg.) for 1 hr. Working up gave the *acetate* of the lactone (VI) (98 mg.), m. p. 266° (decomp.) from cyclopentanone or chloroform–ethanol. Chromatography on alumina (A3) from chloroform–benzene (1:2) followed by crystallisation from chloroform–ethanol did not affect the m. p. (Found: C, 64·1, 64·5; H, 4·6, 4·7. $C_{25}H_{20}O_9$ requires C, 64·65; H, 4·35%), $[\alpha]_D^{27.5}$ −51·7° (*c* 0·81 in $CHCl_3$); $\lambda_{max.}$ ($CHCl_3$) 261 (23,500), 268 (22,600), 299 (19,900), and 342 (8100) mμ; $\nu_{max.}$ ($CHCl_3$) 1736 (acetate and lactone), 1647 (12-ketone), 1629 and 1603 cm.$^{-1}$.

There were n.m.r. resonances at τ 1·05 (1-H), 1·83 (doublet, J = 9 c./sec., 11-H), 3·02 (doublet, J = 9 c./sec., 10-H), 3·14 (4-H), 4·58, 4·67 (7′-vinyl protons), 5·27 (two protons α to acetate on 8′), 6·00, 6·07 (two OMe groups), 7·97 (acetate methyl), near 4·4 (multiplet, 5′-H), near 6·4 (multiplet, 4′-protons).

A second specimen of the acetate was made by treating 6a,12a-dehydroamorphigenin acetate (80 mg.) in glacial acetic acid (2.5 ml.) with pentyl nitrite (0·25 ml.) followed by hydrochloric acid (0·15 ml.) in glacial acetic acid (0·15 ml.). The product (56 mg.) crystallised from chloroform–

ethanol as yellow needles, m. p. and mixed m. p. with the specimen above 264° (decomp.). The identity was checked by infrared spectral comparison.

Alkaline Degradation of Dehydroamorphigenin.—6a,12a-Dehydroamorphigenin (250 mg.) was refluxed with 5% ethanolic potassium hydroxide (25 ml.) for 3 hr. The product was acidified, poured into water, and extracted with ether. The ethereal solution was extracted with 10% sodium carbonate solution, the alkaline extract was acidified, and the *acid* (VII) was collected with ether. It crystallised from aqueous ethanol as needles (130 mg.). Melting-point behaviour was erratic; successive crystallisations gave m. p. 70°, 97—98° (softening at 88·5°), 98—99°, and 88°. When the last material was heated below the m. p., a value of 151—153° (softens at 82—91°) was recorded. The acid gave satisfactory analytical results (Found: C, 62·25; H, 5·6. $C_{23}H_{24}O_9$ requires C, 62·15; H, 5·45%) and had $[\alpha]_D^{26}$ −67·3° (*c* 1·9 in $CHCl_3$); $\lambda_{max.}$ 235 (14,350), 241s (11,850), and 291 (18,700) mμ; $\nu_{max.}$ 1739 (acid), 1634 (ketone, bonded), 1613 (aromatic) cm.$^{-1}$, together with a broad hydroxyl absorption. In the n.m.r. there were resonances at τ 6·18 (two OMe groups), 5·79 (two methylenes), 5·40 (methylene), and 4·78 (vinyl protons). The acid gave a brown ferric reaction.

The acid (50 mg.) and anhydrous sodium acetate (15 mg.) were refluxed in acetic anhydride (0·7 ml. containing 5% of glacial acetic acid) for 10 min. Ethanol (1 ml.) was added and after a further refluxing of 10 min. the volume of the solution was reduced by one-half. A few drops of ethanol were added and the mixture was kept at 0°. 6a,12a-Dehydroamorphigenin acetate, m. p. and mixed m. p. 172·5—174·5°, separated. Identity was confirmed by the infrared spectrum.

Oxidation of the Acid (VII) *to Derric Acid.*—30% Hydrogen peroxide (2 ml.) was added to the acid (VII) (600 mg.) in 5% aqueous potassium hydroxide (25 ml.). The mixture was warmed at 60° for 2 hr., quickly heated to 100°, and then allowed to cool to 20°. Acidification and extraction gave derric acid (113 mg.), m. p. 168°. Identity was confirmed by mixed m. p. (lit.,[37] 171°) and infrared spectrum.

Reduction of Amorphigenin with Sodium Borohydride.—Amorphigenin (1 g.) in ice-cold tetrahydrofuran (20 ml.) and water (5 ml.) was agitated with sodium borohydride (500 mg.) and kept at 20° for 48 hr. Acetone (20 ml.) was added with vigorous stirring and the product was poured into water and extracted with ether. The ether extracts were dried and evaporated to a gum which crystallised from methanol to give the *diol* (IX) (240 mg.), needles, m. p. 148·5° (Found: C, 67·25; H, 6·05. $C_{23}H_{24}O_7$ requires C, 67·0; H, 5·8%), $[\alpha]_D^{25}$ −184·2° (*c* 0·74 in $CHCl_3$); $\lambda_{max.}$ 288 (6600) mμ; $\nu_{max.}$ 1616 (aromatic) cm.$^{-1}$; there were two hydroxyl absorptions in the mull spectrum.

The diol (40 mg.) was refluxed with 20% sulphuric acid (10 ml.) and dioxan (5 ml.) for 1½ hr. The product was poured into water and extracted with chloroform. After being washed and dried, the chloroform extract was treated with charcoal and a little methanol was added. The product was kept at 0° and the solid which separated was not purified further. It had $\lambda_{max.}$ 257 (8950), 264s (8300), 295 (5650), 347s (11,100), 360 (15,510), and 380 (12,750) mμ (cf. Büchi *et al.*[12]).

Oxidation of Amorphigenin with Manganese Dioxide.—Amorphigenin (250 mg.) was shaken with active manganese dioxide (2·5 g.) in chloroform (50 ml.) for 16 hr. Filtration and crystallisation gave yellowish needles (117 mg.) which on chromatography on alumina (A3) from benzene gave colourless needles, m. p. 248—250° (decomp.) (Found: C, 67·95, 67·45; H, 5·1, 5·3. $C_{23}H_{20}O_7$ requires C, 67·65; H, 4·95%), $[\alpha]_D^{17}$ +22·6° (*c* 0·9 in $CHCl_3$). The 8′-*aldehyde* had $\lambda_{max.}$ 234 (15,100), 238s (14,150), and 290 (17,310) mμ; $\nu_{max.}$ 1689 and 1681 (ketone and aldehyde) and 1608 (aromatic) cm.$^{-1}$. There was no hydroxyl absorption in the mull spectra. There were n.m.r. resonances at τ 0·33 (aldehyde), 3·82 (vinyl *cis* to aldehyde), 4·51 (vinyl *trans* to aldehyde), 6·26 and 6·30 (OMe groups).

Hydrogenation of Amorphigenin.—Amorphigenin (500 mg.) was hydrogenated over Adams catalyst (49 mg.) in glacial acetic acid (12·5 ml.) until 75 ml. of hydrogen had been absorbed (18·5°, 757 mm.). Filtration through Celite and evaporation gave a brown gum (466 mg.) and thin-layer chromatography showed the presence of five components (Kieselgel G, chloroform eluant and iodine–chloroform spray). The gum was chromatographed on alumina (A3), all fractions being monitored by thin-layer chromatography. Fractions 1—30 were eluted with benzene, 31—50 with benzene–chloroform (1:1 v/v), and 51—55 with chloroform. Fractions 5 and 6 gave 12-deoxy-6′,7′-dihydrorotenone (14 mg.), m. p. and mixed m. p. with authentic material 171·5° (lit.,[12] 172°) (and infrared comparison). Fractions 16—19 yielded 6′,7′-dihydrorotenone (20 mg.),

[37] LaForge, Haller, and Smith, *J. Amer. Chem. Soc.*, 1930, **52**, 2873.

m. p. and mixed m. p. 210—211° (lit.,[12] 212°) (and infrared comparison). Fractions 23—25 gave an unidentified compound (3 mg.), m. p. 139·5—140·5°. Fractions 34 and 35 gave, after rechromatography, 12-*deoxy*-6′,7′-*dihydroamorphigenin*, needles, m. p. 173° from aqueous methanol (Found: C, 69·5; H, 6·6. $C_{23}H_{26}O_6$ requires C, 69·35; H, 6·6%), $[\alpha]_D^{25}$ −146·4° (*c* 0·94 in $CHCl_3$); $\lambda_{max.}$ 287s (6500) and 290 (6900) mμ; $\nu_{max.}$ 1610 (aromatic), 3534 (hydroxyl) cm.$^{-1}$.

The authors acknowledge with gratitude the help of colleagues. Dr. R. I. Reed provided the mass-spectral molecular weights and Mr. R. Hulme and Mr. M. B. Hursthouse the *X*-ray molecular weights. Professor B. C. L. Weedon arranged for the determination of certain n.m.r. spectra and others were provided by Dr. J. W. Lown who also measured the optical rotatory dispersion curves. Dr. G. O. Aspinall kindly examined the methanolysis product of permethylated amorphin by his recent gas-chromatographic techniques and Mr. A. G. Kenyon (Tropical Products Institute) kept us supplied with *A. fruticosa* seeds. We thank Shell Research Ltd. for a grant, and two of us (J. C. and R. P.) acknowledge the award of D.S.I.R. postgraduate studentships.

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[*Received, June 15th,* 1964.]