Constituents of the Higher Fungi. Part XII.[1] Identification of Involutin as (−)-*cis*-5-(3,4-Dihydroxyphenyl)-3,4-dihydroxy-2-(4-hydroxyphenyl)-cyclopent-2-enone and Synthesis of (±)-*cis*-Involutin Trimethyl Ether from Isoxerocomic Acid Derivatives

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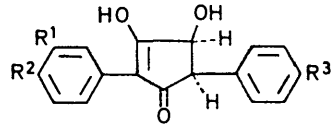
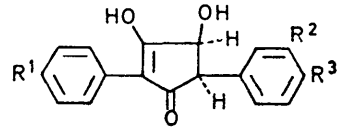
Xerocomic and isoxerocomic acids have been synthesised and converted into the corresponding 2,5-diaryl-4-methoxycyclopent-4-ene-1,3-diones. Reduction of the dione from isoxerocomic acid has given racemic involutin trimethyl ether (IV); this identifies involutin as (III). Isoxerocomic acid is readily oxidised to xerocomorubin; this is compared with the naturally occurring variegatorubin.

In Part IV [2] the isolation and structure determination of involutin [(I) or (III)], a metabolite from the fungus *Paxillus involutus*, was described. The degradative



(I) $R^1 = R^2 = R^3 = OH$
(II) $R^1 = R^2 = R^3 = OMe$



(III) $R^1 = R^2 = R^3 = OH$
(IV) $R^1 = R^2 = R^3 = OMe$

evidence on the basis of which structures (I) and (III) were proposed did not distinguish between the two positional isomers, and syntheses of both appeared to be the best way of establishing the structure. A synthesis based on the condensation of the appropriate substituted dibenzyl ketone with diethyl oxalate [3] is inapplicable in this case since reduction of the unsymmetrical triketone would give rise to a mixture of two isomers of unknown constitution. An unambiguous synthesis, based on the possible biosynthetic route, involves the rearrangement of the appropriate unsymmetrically substituted pulvinic acid derivatives.

The aromatic hydroxy-substitution pattern in involutin is identical with that occurring in the fungus metabolite xerocomic acid,[4] and this compound is the obvious starting material for the proposed synthesis. However, the orientation of the aryl residues in xerocomic acid is also unknown and it was first necessary to synthesise both the isomers (XVIII) and (XIX).

Condensation of ethyl 3-cyano-3-(4-methoxyphenyl)-pyruvate with 3,4-dimethoxyphenylacetonitrile in the presence of sodium hydride gave a dinitrile, m.p. 234—237° (lit.,[4] 221—222°). Hydrolysis of this with sulphuric acid and treatment of the product with acetic anhydride gave 3,4,4′-trimethoxypulvinic acid lactone (V). Demethylation of this dilactone with hydrogen iodide produced two isomeric acids, one red and the other orange. The yield of each depends on the reflux time; the red, more soluble compound is the sole product with a reflux time of more than 1 h but hydrolysis for not more than 20—25 min produced a mixture of the two compounds which were separated by crystallisation. Hydrolysis with hydrogen bromide in acetic acid produced low yields of only the red isomer. The isomeric acids, $C_{18}H_{12}O_8$, may be distinguished by a difference in m.p. [red isomer 295° (decomp.), orange isomer 300—305°] and a marked difference in the aromatic proton splitting pattern in the ^1H n.m.r. spectrum. Solutions of the red isomer yield a blue colouration on addition of dilute ammonia solution; the orange isomer yields a yellow colouration with this reagent. Both compounds give xerocomic acid lactone triacetate, $C_{24}H_{16}O_{10}$, with sulphuric acid and acetic anhydride, and methylation of the red and the orange compounds with diazomethane gave a bright yellow and a bright fluorescent yellow tetramethoxy-methyl ester, m.p. 148 and 151° [(XIV) and (XVI)], respectively.

Spectral data

	^1H N.m.r. [τ ($CD_3 \cdot CO_2D$)]	$\lambda_{max.}$(EtOH)/nm (log ε)
(−)-*cis*-Involutin trimethyl ether (IV) from naturally occurring involutin	1·98 (1H), 2·07 (1H), 2·92—3·26 (5H), 5·00 (1H, d), 5·68 (1H, d) (*J* 7 Hz), 6·14, 6·15, 6·17 (each 3H)	226infl (4·08) 260 (4·10) 276 (4·12)
(−)-*trans*-Involutin trimethyl ether from naturally occurring involutin	†, 1·87 (1H), 1·96 (1H), 2·94—3·23 (5H), 5·27 (1H, d), 6·38 (1H, d) (*J* 3 Hz), 6·14, 6·15, 6·16 (each 3H)	226infl (4·24) 261sh, (4·24) 277 (4·28)
Synthetic (±)-*cis*-involutin trimethyl ether (IV)	1·98 (1H), 2·07 (1H), 2·92—3·26 (5H), 5·00 (1H, d), 5·68 (1H, d) (*J* 7 Hz), 6·14, 6·15, 6·17 (each 3H)	226infl (4·08) 260 (4·11) 276 (4·13)
Synthetic (±)-*cis*-isoinvolutin trimethyl ether (II)	2·31—3·08 (7H), 5·02 (1H, d), 5·71 (1H, d) (*J* 7 Hz), 6·10 (6H), 6·16 (3H)	225sh, (4·24) 259 (4·13) 274infl (4·08) 281infl (4·06) 292infl (3·96)

† In $(CD_3)_2CO$.

In methylation of the red and the orange compounds with diazomethane gave a bright yellow and a bright fluorescent yellow tetramethoxy-methyl ester, m.p. 148 and 151° [(XIV) and (XVI)], respectively.

Although it has been reported that fully methylated

[1] Part XI, P. C. Beaumont and R. L. Edwards, *J. Chem. Soc.* (*C*), 1971, 2582.
[2] R. L. Edwards, G. C. Elsworthy, and N. Kale, *J. Chem. Soc.* (*C*), 1967, 405.
[3] L. Claisen and T. Ewan, *Annalen*, 1895, **284**, 245.
[4] W. Steglich, W. Furtner, and A. Prox, *Z. Naturforsch.*, 1968, **23b**, 1044.

pulvinic acid derivatives can be degraded by ozonolysis,[5] we were unable to degrade the fully methylated xerocomic acids successfully in this way and it became

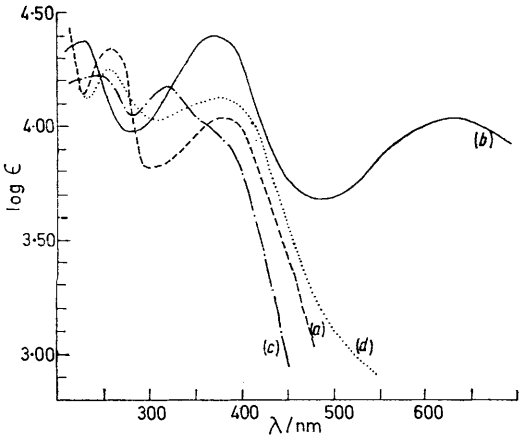


FIGURE 1 U.v. spectra of (*a*) xerocomic acid in 10% ethanol, (*b*) ditto plus 2 drops 2N-NH_4OH, (*c*) isoxerocomic acid in 10% ethanol, (*d*) ditto plus 2 drops 2N-NH_4OH

necessary to relate the tetramethoxy-methyl esters to the trimethoxy-methyl esters derived from trimethoxypulvinic acid lactone (V). Trimethoxypulvinic acid lactone was methanolysed with methanolic potassium hydroxide; fractional crystallisation and column chromatography separated two isomeric trimethoxy-methyl esters, (VII and XI), $C_{22}H_{20}O_8$, which were distinguished by differences in m.p. (177 and 168°) and in u.v. spectrum. Methylation of the higher-melting ester (VII) with diazomethane gave the tetramethoxy-methyl ester (XIV), m.p. 148°; similarly the lower-melting ester (XI) gave (XVI), m.p. 151°. The products were identical with those obtained from the isomeric acids.

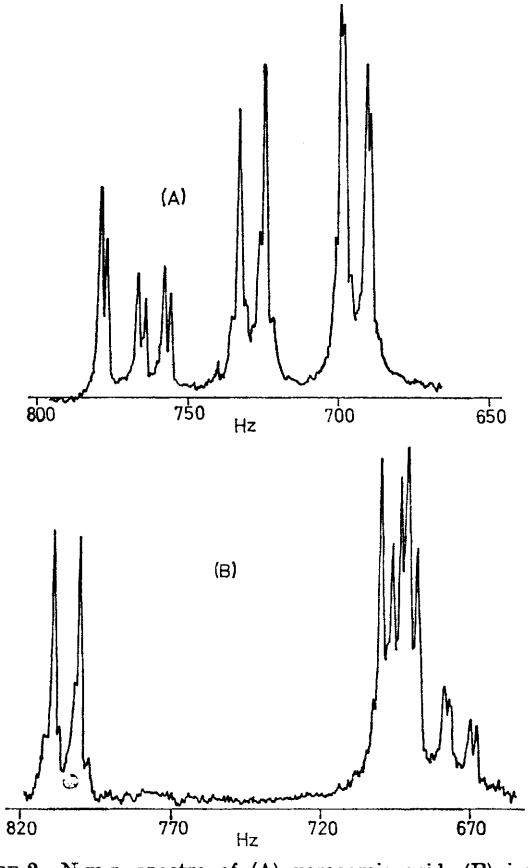


FIGURE 2 N.m.r. spectra of (A) xerocomic acid, (B) isoxerocomic acid [frequency 100 MHz, sweep width 270 Hz, solvent $(CD_3)_2CO$ + 2 drops D_2O]

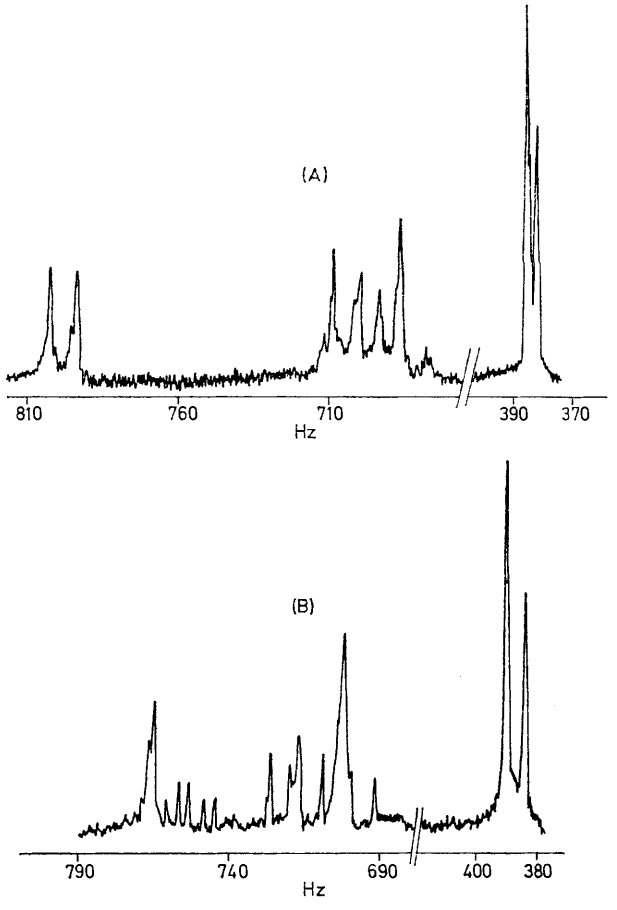


FIGURE 3 N.m.r. spectra of (A) involutin trimethyl ether, (B) isoinvolutin trimethyl ether (frequency 100 MHz, sweep width 270 Hz, solvent CD_3CO_2D)

Ozonolysis of (VII) gave methyl 4-methoxybenzoylformate and (XI) gave methyl 3,4-dimethoxybenzoylformate. This established the identity of (VII) as methyl 3,4,4′-trimethoxypulvinate and that of (XI) as methyl 3′,4′,4-trimethoxypulvinate, and established the structures of xerocomic acid and isoxerocomic acid as (XVIII) and (XIX), respectively.

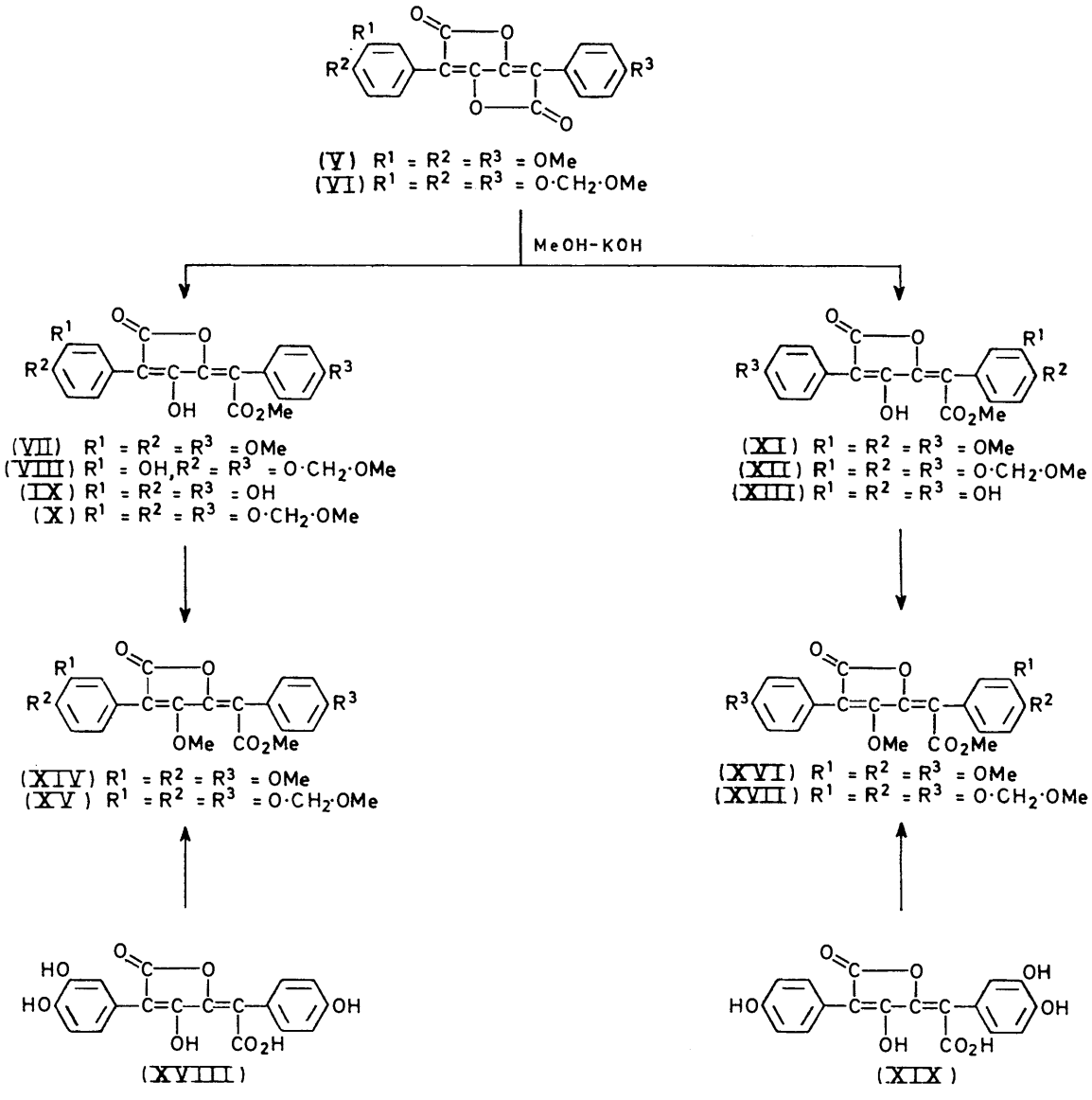
Differences in colour reaction with alkali between the isomers of methyl dihydroxypulvinate[6] have been explained in terms of steric hindrance produced by the ester group, and this explanation was supported by differences in u.v. absorption at 336 and 390 nm between

[5] P. Karrer, K. A. Gehrckens, and W. Heuss, *Helv. Chim. Acta*, 1926, **9**, 446.

[6] P. C. Beaumont, R. L. Edwards, and G. C. Elsworthy, *J. Chem. Soc.* (*C*), 1968, 2968.

the two isomers. A comparison of the long wavelength u.v. maxima (in ethanol) of isoxerocomic acid (391 nm) and methyl isoxerocomate (XIII) (397 nm) with the maxima of xerocomic acid (406 nm) and methyl xerocomate (IX) (397 nm) shows that loss of coplanarity within the molecule is not an important factor. Direct conjugation between the catechol and the enolic hydroxy-group must be the primary requisite for the development of the blue colour.

Other naturally occurring unsymmetrically substituted pulvinic acids exist in only one form, and in order to establish whether naturally occurring xerocomic acid existed as a mixture the crude extracts from *Gomphidius rutilus* were re-examined;[1] the crude gummy extract was purified by t.l.c. A portion of the chromatographically pure pigment was recrystallised from formic acid and gave xerocomic acid as bright red needles, $C_{18}H_{12}O_8$, m.p. 295° (decomp.). The position of the i.r. and u.v. maxima both in 10% ethanol and in alkali established the identity of the natural pigment with 3,4,4′-trihydroxypulvinic acid (XVIII). The aromatic splitting pattern in the ^1H n.m.r. spectrum of naturally occurring xerocomic acid was identical with that of the synthetic compound and showed no absorption characteristic of isoxerocomic acid. The possibility that recrystallisation had removed small quantities of the iso-acid was discounted when methylation of the chromatographically pure pigment gave methyl 3,4,4′-trimethoxypulvinate methyl ether (XIV) as the only product.

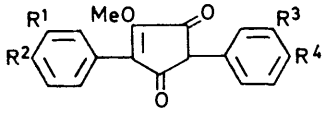


Owing to the instability of 3,4-dihydroxypulvinic acid derivatives towards alkali, a synthesis of involutin by alkaline rearrangement must involve protection of the hydroxy-groups. The successful use of the methoxymethyl group in other syntheses *e.g.* of aurantiacin,[7]

[7] R. L. Edwards and N. Kale, *J. Chem. Soc.*, 1964, 4084.

prompted its use as a protecting group in this synthesis. However, the synthesis of involutin trimethyl ether from the methylated derivatives was investigated first. Treatment of methyl *O*-methyl-3,4,4′-trimethoxypulvinate (XIV) with 4% methanolic potash resulted in rapid solution and the development of an intense purple colouration; acidification gave the cyclopentenone (XX) in good yield. Similar treatment of methyl *O*-methyl-3′,4′,4-trimethoxypulvinate (XVI) resulted in a much slower reaction and the separation of a colourless potassium salt which gave unchanged starting material on acidification. Repeated treatment with alcoholic alkali gave the required cyclopentenone (XXII).

Reduction of both compounds (XX) and (XXII) with sodium borohydride resulted in the simultaneous removal of the enolic ether group and gave the colourless trimethyl ethers (II) and (IV), respectively.



(XX) $R^1 = R^2 = R^4 = OMe, R^3 = H$
(XXI) $R^1 = R^2 = R^4 = O{\cdot}CH_2{\cdot}OMe, R^3 = H$
(XXIII) $R^2 = R^3 = R^4 = OMe, R^1 = H$
(XXIII) $R^2 = R^3 = R^4 = O{\cdot}CH_2{\cdot}OMe, R^1 = H$

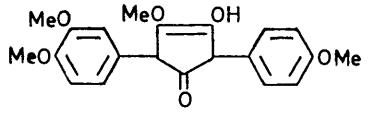
The cyclopentenones (II) and (IV) may be distinguished by differences in m.p. (199—202 and 204—207°, respectively) and in u.v. and ^{1}H n.m.r. spectra. The most interesting difference is seen in the ^{1}H n.m.r. aromatic proton pattern; the ether (II) shows a close seven-proton multiplet between τ 2·31 and 3·08 whereas the ether (IV) shows a five-proton multiplet between τ 2·92 and 3·26 together with a low field two-proton doublet at τ 2·02. Involutin and all its derivatives show a similar low-field doublet.

The mass spectra of both compounds show molecular ions at *m/e* 356 with more intense ions at *m/e* 338 ($M - H_2O$). In the spectrum of (II) the ion at *m/e* 338 is the base peak but in (IV) the base peak appears at *m/e* 191. The ether (II) shows only a weak *m/e* 191 peak but a strong peak at *m/e* 161 (94%). This is the most important difference between the two spectra.

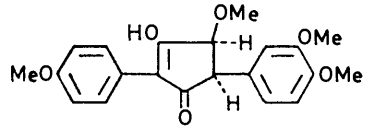
Involutin trimethyl ether is recorded [2] as a waxy solid, m.p. 159—161°, which in acetone solution shows methoxy-absorption in the ^{1}H n.m.r. at τ 6·16, 6·20, and 6·23 and doublet methine signals at τ 5·11 and 5·91. The m.p. of this compound and the position of these n.m.r. bands do not coincide with those of either of the synthetic trimethyl ethers, but a rigorous comparison could not be made because the synthetic compounds were insufficiently soluble in acetone. The non-crystalline nature of involutin trimethyl ether and the absence of recorded u.v. and mass spectra prompted a repeat preparation of the compound. In the presence of an excess of alkali a colourless trimethyl ether, $C_{20}H_{20}O_6$, m.p. 180—185°, $[\alpha]_D^{20}$ −30·6 (EtOH) was produced. The m.p., i.r. absorption, and ^{1}H n.m.r. spectrum are different from those reported for involutin trimethyl ether and the appearance of the methine protons as a pair of doublets (*J* 3 Hz) immediately characterised this compound as the *trans*-isomer of involutin trimethyl ether. By careful control of the alkalinity during the methylation, a gelatinous product was obtained, which crystallised with difficulty and much loss from absolute ethanol, to yield *cis*-involutin trimethyl ether, m.p. 166—168°, $[\alpha]_D^{20}$ (EtOH) −27·5°, as colourless irregular plates. A comparison of the u.v., ^{1}H n.m.r., and mass spectra established its identity with (IV) and hence the structure of involutin as (III).

The i.r. spectrum of the trimethyl ether of natural involutin is slightly different from the spectrum of the synthetic ether. These differences in spectra, in m.p., and in solubility between the synthetic and naturally derived ethers are consistent with the formation of a racemic compound in the case of the synthetic trimethyl ether. Such a compound would be expected to show different properties from the enantiomerically pure trimethyl ether of natural involutin.

The tetramethyl ether produced by the action of methyl iodide and potassium carbonate on involutin has previously been assigned structure (XXIV); [2] this assignment was based on the absence of doublets and the presence of a two-proton singlet at τ 5·50 in the 60 MHz ^{1}H n.m.r. spectrum. However, the 100 MHz spectrum of the compound distinguishes the methine resonance as a closely spaced pair of one-proton doublets at τ 5·43 and 5·54 (*J* 7 Hz) and establishes the structure as that of the enol (XXV). This is consistent with the solubility of this ether in caustic alkali.



(XXIV)



(XXV)

The separation of xerocomic and isoxerocomic acids appeared to facilitate the synthesis of involutin itself and also its positional isomer. However, attempts to obtain pure methoxymethoxy-xerocomic and isoxerocomic acids gave uncrystallisable oils. The ready degradation of these oils to more polar products caused this route to be abandoned in favour of one involving the 3,4,4′-tris-(methoxymethoxy)pulvinic acid lactone (VI). The mixture of xerocomic and isoxerocomic acids was methoxymethylated and the oily product was converted into the crystalline pulvinic acid lactone (VI) with acetic anhydride. Methanolysis of the lactone with methanolic 2% alkali at room temperature, or by heating with methanol, gave a mixture of two esters. Separation by chromatography gave a crystalline ester and a yellow oil which could not be crystallised. Acidic hydrolysis of

the crystalline ester, $C_{25}H_{26}O_{11}$, m.p. 138—140°, gave an orange crystalline phenolic ester, $C_{19}H_{14}O_8$, which on methylation yielded methyl *O*-methyl-3′,4′,4-trimethoxypulvinate (XVI); thus the crystalline methanolysis product was identified as methyl 3′,4′,4-tris(methoxymethoxy)pulvinate (XII) and the hydrolysis product as methyl isoxerocomate (XIII).

The non-crystalline oil (VIII) showed lactone and chelated ester carbonyl absorption at 1780 and 1679 cm^{-1}, but analysis and high resolution mass measurement indicated a molecular formula $C_{23}H_{22}O_{10}$. The ^1H n.m.r. spectrum showed the presence of only two methoxymethyl groups. The presence of a free phenolic hydroxy-group in this compound was confirmed when methylation with diazomethane gave an oily dimethyl ether, $C_{25}H_{26}O_{10}$. Acetylation with acetic anhydride in pyridine gave a monoacetate which still contained a free enolic hydroxy-group [$\nu_{max.}$ 1671 cm^{-1} (chelated ester)]. The evidence for the identity of the non-crystalline ester was obtained by mass spectrometry: * 3,4,4′-tris(methoxymethoxy)pulvinic acid lactone shows a base peak at *m/e* 394 ($M - CH_3{\cdot}O{\cdot}CH_2{\cdot}O{\cdot}CH_3$). Similarly the crystalline ester (XII) first lactonises ($M^+ - CH_3{\cdot}OH \longrightarrow$ *m/e* 470) and then expels $CH_3OCH_2OCH_3$ to give a base peak at *m/e* 394. The $CH_3{\cdot}O{\cdot}CH_2{\cdot}O{\cdot}CH_3$ unit arises from the 3,4-bis(methoxymethyl) group. The non-crystalline ester also lactonises, to give an ion *m/e* 426 ($M^+ - CH_3{\cdot}OH$) but shows no further loss of 76 mass units. This suggests that the non-crystalline ester does not possess a 3,4-bis(methoxymethoxy)phenyl group. The fact that one methanolysis product is a stable substituted ester implies that the labile methoxymethyl group (and hence the free hydroxy-group) occupies a *meta*-position, since this is the only position which is differently influenced in the two isomeric compounds.

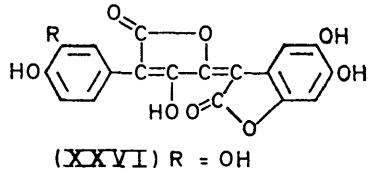
Final proof of the identity of the non-crystalline ester was obtained when hydrolysis with dilute sulphuric acid gave a crystalline phenolic ester (IX), $C_{19}H_{14}O_8$, m.p. 258—261°, which with diazomethane gave methyl *O*-methyl-3,4,4′-trimethoxypulvinate (XIV).

The ester (VIII) was remethoxymethylated and gave a mixture of methyl tetrakis(methoxymethoxy)pulvinate, unchanged starting material, and the required methyl 3,4,4′-tris(methoxymethoxy)pulvinate (X). Decomposition of the latter prevented the ester from being obtained analytically pure but methylation of the freshly chromatographed sample with diazomethane gave crystalline methyl *O*-methyl-3,4,4′-tris(methoxymethoxy)pulvinate (XV), $C_{26}H_{28}O_{11}$, m.p. 106—109°. Similarly, methylation of the crystalline ester gave methyl *O*-methyl-3′,4′,4-tris(methoxymethoxy)pulvinate (XVII), m.p. 91—93°. With methanolic 4% potassium hydroxide solution the 3,4,4′-isomer gave the characteristic violet solution which on acidification gave the cyclopentenedione (XXI) $C_{24}H_{26}O_9$. Similarly the 3′,4′,4-compound yielded (XXIII). The diketones (XXI) and (XXIII) are distinguished by differences in m.p. (95 and 64°, respectively), i.r. absorption, and multiplicity in the ^1H n.m.r. spectrum.

Because of the small quantities of synthetic intermediates available at this stage and the low yields which had been experienced during the borohydride reduction stage of the involutin trimethyl ether synthesis, the borohydride reduction products were not isolated but were hydrolysed *in situ*, and the hydrolysate was compared with authentic involutin. The product from the reduction and hydrolysis of (XXIII) was a mixture of three compounds. Two of these (R_F 0·97 and 0·82 on silica) represented the bulk of the product and presumably corresponded to involutin-type compounds in which the enolic hydroxy-group had remained methylated. A third compound (R_F 0·65) corresponded in R_F value to authentic involutin. Chromatographic examination of the reduction and hydrolysis products of diketone (XXI) showed the presence of only products of high R_F (0·95—0·98). A large proportion of this product ran as a yellow spot and it was apparent that the borohydride reduction had been largely unsuccessful.

The occurrence of a compound with an R_F value identical with that of authentic involutin as a product derived from the 3′,4′,4-isomeric series supports the trimethyl ether synthesis and identifies involutin as 5-(3,4-dihydroxyphenyl)-3,4-dihydroxy-2-(4-hydroxyphenyl)cyclopent-2-enone (III).

A number of boletes contain the red colouring matter variegatorubin (XXVI) and Steglich *et al.*[8] were able to synthesise this compound from variegatic acid by oxidation with hydrogen peroxide in the presence of a copper catalyst. The reaction presumably involves hydroxylation of the aromatic ring adjacent to the carboxy-group followed by spontaneous lactonisation. In view of the substitution pattern in natural xerocomic acid it is not surprising that Steglich was unable to oxidise this compound to a calycin analogue. However, isoxerocomic acid should be capable of being oxidised in this way. Reaction with hydrogen peroxide in the presence of ammonium tungstate resulted in a vigorous reaction and the formation of the dark red xerocomorubin (XXVII), $C_{18}H_{10}O_8$, m.p. 285° (decomp.). Variegatic acid was similarly oxidised to variegatorubin but xerocomic acid was unaffected by this reagent.



(XXVI) R = OH
(XXVII) R = H

Xerocomorubin shows carbonyl absorption at 1750sh and 1722 cm^{-1} (unchelated and chelated lactone respectively) and the long wavelength u.v. absorption at 497 nm corresponds to a bathochromic shift of 106 nm in comparison with the long wavelength absorption of

* Proposals supported by high resolution measurements.

[8] W. Steglich, W. Furtner, and A. Prox, *Z. Naturforsch.*, 1969, **24b**, 941.

isoxerocomic acid. Similar large shifts have been noted between variegatorubin and variegatic acid and between calycin and pulvinic acid.

Acetylation of xerocomorubin with acetic anhydride and sulphuric acid gives a triacetate, $C_{24}H_{16}O_{11}$, and with diazomethane a tetramethyl ether, $C_{22}H_{18}O_8$, is produced. The spectra of the latter compound are similar to those of *O*-methylcalycin.

Since variegatorubin has been found in members of the Boletaceae it is reasonable to expect that xerocomorubin might also occur naturally. The alcoholic extracts of the red cap skins of *Boletus rubellus* Krombh. are of a similar colour to those of variegatorubin and xerocomorubin, but a t.l.c. comparison of the fungus extract with solutions of the two synthetic pigments showed the presence of only variegatorubin.

The obvious structural relationship between leucomelone, xerocomic acid, and involutin poses an interesting biosynthetic problem. If involutin is derived *via* compounds of the pulvinic acid type, as seems likely, then its logical precursor will be isoxerocomic acid rather than the naturally occurring isomer. Presumably if the metabolic balance requires the presence of trihydroxypulvinic acid then it produces the stable isomer, xerocomic acid.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 237 spectrophotometer, u.v. spectra on a Unicam SP 800 spectrophotometer, ^{1}H n.m.r. spectra on a J.E.O.L. JNM-MH-100 spectrometer except where stated otherwise (with tetramethylsilane as an internal standard), and the mass spectra on an A.E.I. MS9 spectrometer. All thin layer (t.l.c.), preparative layer (p.l.c.), and column chromatography was done on Merck kieselgel $PF_{256+366}$; preparative layers consisted of silica gel (16 g) on 20 × 20 cm glass plates. Solvent systems used in chromatography were (1) benzene–ethyl formate–formic acid (13 : 5 : 4); (2) benzene–acetic acid (95 : 15).

2-(3,4-Dimethoxyphenyl)-5-(4 methoxyphenyl)-3,4-dioxoadiponitrile.—Ethyl 3-cyano-3-(4-methoxyphenyl)pyruvate (228 g, 0·92 mol) in dry dimethoxyethane (800 ml) was added dropwise over 6 h to a stirred mixture of 3,4-dimethoxyphenylacetonitrile (163·4 g, 0·92 mol) and sodium hydride (171 g) in dry dimethoxyethane (800 ml) at −10°. Nitrogen was bubbled through the mixture during the addition, after which the temperature was allowed to rise and the mixture was set aside overnight. The solid mixture was carefully diluted with water (4 l) and washed well with ether. Acidification of the aqueous layer with acetic acid gave a red precipitate which was separated, washed with water and ether, and dried. Crystallisation from acetic acid gave *2-(3,4-dimethoxyphenyl)-5-(4-methoxyphenyl)-3,4-dioxoadiponitrile* (217 g, 62%) as golden yellow plates, m.p. 234—237° (lit.,[4] 221—222°) (Found: C, 66·5; H, 4·8; N, 7·5. $C_{21}H_{18}N_2O_5$ requires C, 66·7; H, 4·8; N, 7·4%); $\nu_{max.}$ (KBr) 2840 and 2224 cm^{-1}.

3,4,4′-Trimethoxypulvinic Acid Lactone (V).—A mixture of 2-(3,4-dimethoxyphenyl)-5-(4-methoxyphenyl)-3,4-dioxoadiponitrile (160 g), acetic acid (1500 ml), sulphuric acid (750 ml; *d* 1·84), and water (1110 ml) was refluxed. The dinitrile dissolved almost immediately and after a few minutes an orange crystalline solid was deposited. After 1 h the mixture was cooled and the crystalline solid filtered off, washed with acetic acid and ether, and dried. Heating the compound under reflux with acetic anhydride (1 l) and cooling gave *3,4,4′-trimethoxypulvinic lactone* (107 g) as orange needles, m.p. 230—231° (decomp.) (Found: C, 66·3; H, 4·2; OCH_3, 24·8. $C_{21}H_{16}O_7$ requires C, 66·3; H, 4·2; OCH_3, 24·5%); $\nu_{max.}$ (KBr) 1821 and 1780sh cm^{-1}.

A similar hydrolysis of the dinitrile (m.p. 218—220°) prepared by the method of Asano and Kameda [9] gave a dark red crystalline product on acidification. T.l.c. examination in solvent (2) showed the presence of four spots corresponding to authentic samples of 4,4′-dimethoxypulvinic acid (R_F 0·30), 3,4,4′-trimethoxypulvinic acid (0·14), 3′,4′,4-trimethoxypulvinic acid (0·10), and 3,3′,4,4′-tetramethoxypulvinic acid (0·02).

Methanolysis of 3,4,4′-Trimethoxypulvinic Acid Lactone.—A suspension of the lactone in methanolic potassium hydroxide (250 ml; 2%) was stirred vigorously until dissolution was complete (15 min). The orange solution was poured into water (1 l) and the solution acidified with acetic acid; the bright orange precipitate was filtered off, washed with water, and dried. Crystallisation from ethanol (200 ml) gave orange needles of *methyl 3,4,4′-trimethoxypulvinate* (VII), which crystallised from benzene as orange tablets (660 mg), m.p. 177° (Found: C, 64·1; H, 4·9. $C_{22}H_{20}O_8$ requires C, 64·1; H, 4·85%); $\nu_{max.}$ (KBr) 3500, 2590, 1771 (C=O), and 1675 cm^{-1} (chelated C=O); $\lambda_{max.}$ (EtOH) 270·5, 310infl, 326infl, and 396 nm (log ε 4·04, 3·95, 3·87, and 3·87); τ ($CDCl_3$) 2·04—3·02 (7H) and 6·00, 6·02, 6·05, and 6·09 (each 3H).

Evaporation of the ethanolic mother liquor gave orange needles (2·20 g), which when applied to a column of silica gel (100 × 2·5 cm) in solvent (2) separated into two closely spaced yellow bands. The first band gave methyl 3,4,4′-trimethoxypulvinate (305 mg). The second band gave a residue which crystallised from benzene to give *methyl 3′,4′,4-trimethoxypulvinate* (XI) (1 g) as clusters of yellow plates, m.p. 168° (Found: C, 64·1; H, 4·8. $C_{22}H_{20}O_8$ requires C, 64·1; H, 4·85%); $\nu_{max.}$ (KBr) 3450, 2560, 1769 (C=O), and 1672 cm^{-1} (chelated C=O); $\lambda_{max.}$ (EtOH) 240, 266, 276infl, and 394 nm (log ε 4·24, 4·22, 4·18, and 4·09); τ ($CDCl_3$) 1·72 (1H), 1·81 (1H), 2·91—3·15 (5H), 6·04 (3H), 6·07 (6H), and 6·11 (3H).

Ozonolysis of Methyl 3,4,4′-Trimethoxypulvinate.—Ozonised oxygen was passed through a solution of 3,4,4′-trimethoxypulvinate (0·5 g) in anhydrous chloroform (200 ml) at −20° until the orange colour faded (2 h). The solvent was removed under reduced pressure and the residue was hydrogenated in the presence of palladium–charcoal (500 mg; 5%) in anhydrous ethyl acetate solution until uptake of hydrogen was complete. The residue was dissolved in ether and the solution shaken with sodium hydrogen carbonate solution and then with water; it was finally dried and evaporated. The almost colourless residual oil was identified as methyl 4-methoxybenzoylformate by spectral comparison with authentic material.

Similarly methyl 3′,4′,4-trimethoxypulvinate gave methyl 3,4-dimethoxybenzoylformate.[6]

Methyl O Methyl-3,4,4′-trimethoxypulvinate (XIV).—Methyl 3,4,4′-trimethoxypulvinate (400 mg) was treated with an excess of ethereal diazomethane. After 6 h the excess of reagent was destroyed with acetic acid and the solvent was evaporated off under reduced pressure.

[9] M. Asano and Y. Kameda, *Ber.*, 1934, **67b**, 1522.

Crystallisation of the residue from alcohol gave *methyl* O-*methyl*-3,4,4′-*trimethoxypulvinate* (250 mg) as small cadmium-yellow needles, m.p. 146—148° (Found: C, 64·9; H, 5·2. $C_{23}H_{22}O_8$ requires C, 64·8; H, 5·2%); $\nu_{max.}$ (KBr) 1764 (C=O, lactone) and 1726 cm^{-1} (C=O, ester); $\lambda_{max.}$ (EtOH) 240 and 361 nm (log ε 4·37 and 4·49); τ ($CDCl_3$) 2·32—3·14 (7H), 6·10 (9H), and 6·18 and 6·23 (each 3H).

Similarly, methylation of methyl 3′,4′,4-trimethoxypulvinate gave *methyl* O-*methyl*-3′,4′,4-*trimethoxypulvinate* (XVI) as yellow rods (from ethanol), m.p. 150—151° (Found: C, 64·8; H, 5·3%); $\nu_{max.}$ (KBr) 1764 (C=O, lactone) and 1726 cm^{-1} (C=O, ester); $\lambda_{max.}$ (EtOH) 241, 265infl, and 370 nm (log ε 4·31, 4·12, and 4·45); τ ($CDCl_3$) 2·46—3·19 (7H), 6·12 (3H), 6·14 (6H), and 6·21 and 6·27 (each 3H).

Attempted ozonolysis of these fully methylated compounds for 2 h gave only the unchanged ethers.

5-(3,4-*Dimethoxyphenyl*)-4-*methoxy* 2-(4-*methoxyphenyl*)-*cyclopent*-4-*ene*-1,3-*dione* (XX).—Methyl *O*-methyl-3,4,4′-trimethoxypulvinate (747 mg) was stirred (20 min) with methanolic potassium hydroxide solution (40 ml; 4%). The solid dissolved within 3 min and the solution developed a violet colouration which persisted throughout the reaction. The solution was acidified with acetic acid and the solvent removed under reduced pressure. The solid residue was washed with hot water, filtered off, dried and crystallised from ethanol to give the *dione* (XX) (226 mg) as yellow leaflets, m.p. 151—153° (Found: C, 68·5; H, 5·4. $C_{21}H_{20}O_6$ requires C, 68·5; H, 5·4%); $\nu_{max.}$ (KBr) 1728, 1683sh, and 1672 cm^{-1}; $\lambda_{max.}$ (EtOH) 220sh, 257, 269·5infl, 325infl, and 366 nm (log ε 4·34, 4·19, 4·11, 3·75, and 4·01); τ ($CDCl_3$) 2·24—3·20 (7H), 5·60 (3H), 6·00 (1H), 6·11 (6H), and 6·26 (3H).

2-(3,4-*Dimethoxyphenyl*)-4-*methoxy*-5-(4-*methoxyphenyl*)-*cyclopent*-4-*ene*-1,3-*dione* (XXII).—Methyl *O*-methyl-3′,4′,4-trimethoxypulvinate (900 mg) was stirred with methanolic potassium hydroxide (40 ml; 4%) at room temperature. The solid dissolved over 5 min and the solution developed a dull red-violet colouration. After 10 min the solution deposited the *potassium salt* of *dimethyl* 4-(3,4-*dimethoxyphenyl*)-3-*hydroxy*-2-*methoxy*-1-(4-*methoxyphenyl*)*buta*-1,3-*diene*-1,4 *dicarboxylate* as pale yellow needles (600 mg), m.p. 230° (decomp.) (softens at 154°) (Found: C, 58·0; H, 5·1; K, 6·8. $C_{24}H_{25}KO_9$ requires C, 58·05; H, 5·0; K, 7·9%); $\nu_{max.}$ (KBr) 1730 and 1687 cm^{-1}; τ [$(CD_3)_2CO$] 2·30—3·11 (7H) and 6·07, 6·08, 6·09, 6·10, 6·12, and 6·16 (each 3H).

The filtrate was acidified with acetic acid and the crystalline precipitate crystallised from ethanol to give the *dione* (XXII) (123 mg) as pale yellow, hair-like needles, m.p. 163—165° (Found: C, 68·4; H, 5·4. $C_{21}H_{20}O_6$ requires C, 68·5; H, 5·4%); $\nu_{max.}$ (KBr) 1724 and 1674 cm^{-1}; $\lambda_{max.}$ (EtOH) 241infl, 251, and 354 nm (log ε 4·31, 4·35, and 4·24); τ ($CDCl_3$) 1·92 (1H), 2·01 (1H), 3·01—3·38 (5H), 5·62 (3H), 6·02 (1H), and 6·18 (9H).

The salt (360 mg) was re-suspended in methanolic potassium hydroxide solution (40 ml; 4%). After 2 h the unchanged salt (104 mg) was filtered off and the filtrate acidified to yield the dione (61 mg), identical with that just described. Acidification of an aqueous solution of the potassium salt with acetic acid gave unchanged starting material.

(±)-cis-5-(3,4-*Dimethoxyphenyl*)-3,4-*dihydroxy*-2-(4-*methoxyphenyl*)*cyclopent*-2-*en*-1-*one* (IV).—A solution of the dione (XX) (275 mg) in ethanol (40 ml) was added dropwise to a stirred solution of sodium borohydride (140 mg) in water (8 ml) at room temperature. After 1 h the mixture was diluted with water (60 ml), acidified with hydrochloric acid (2N), and set aside overnight. The mixture was extracted with ether (3 × 40 ml) and the extract was washed with water and dried (Na_2SO_4); evaporation gave a pale yellow gum. Trituration with chloroform and recrystallisation of the crystalline solid from alcohol gave the *cyclopentone* (IV) (20 mg) as lustrous leaflets, m.p. 204—207° (Found: C, 67·3; H, 5·75. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·6%); $\nu_{max.}$ (KBr) 3410, 3700—2100, and 1640sh cm^{-1}.

Similarly, reduction of the dione (XXII) gave the *cyclopentenone* (II) (15 mg) as needles (from ethanol), m.p. 199—202° (Found: C, 67·4; H, 5·6%); $\nu_{max.}$ (KBr) 3410, 3700—2100, and 1636sh cm^{-1}.

(−)-trans-*Involutin Trimethyl Ether*.—Aqueous sodium hydroxide solution (7·5 ml; 2N) was added to a mixture of involutin (300 mg) and dimethyl sulphate (1·5 ml) in methanol (5 ml) under nitrogen at room temperature. The gummy solid which separated after 10 min was redissolved by addition of further alkali (10 ml). After 20 h the mixture was acidified with hydrochloric acid (2N) and the flocculent precipitate was extracted with ethyl acetate (2 × 20 ml). The extracts were dried and evaporated and the residual solid was dissolved in chloroform (0·6 ml) and set aside. The crystalline solid was separated and recrystallised three times from chloroform to give trans-*involutin trimethyl ether* (57 mg) as long needles, m.p. 180—185°, $[\alpha]_D^{20}$ (EtOH) −30·6° (Found: C, 67·1; H, 5·7; OMe, 26·0. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·6; OMe, 26·1%); $\nu_{max.}$ (KBr) 3450 (OH), 1654sh, and 1595 cm^{-1}.

(−)-cis-*Involutin Trimethyl Ether* (IV).—Involutin (300 mg) was methylated similarly; the solution was maintained alkaline by the periodic dropwise addition of aqueous sodium hydroxide (2N). After 7 h the mixture was acidified with hydrochloric acid (2N) and extracted with ethyl acetate (3 × 20 ml). The extracts were dried and evaporated and the residual solid dissolved in chloroform (0·6 ml) was set aside. The waxy solid which separated was crystallised from ethyl acetate (× 2) and then from absolute ethanol at 0° to give cis-*involutin trimethyl ether* (15 mg) as irregular plates, m.p. 166—168° (lit.,[2] 159—161°), $[\alpha]_D^{20}$ (EtOH) −27·5° (Found: C, 67·4; H, 5·6. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·6%); $\nu_{max.}$ (KBr) 3410, 3600—2200, 1660sh, 1600sh, and 1586 cm^{-1}.

3,4,4′-*Trihydroxypulvinic Acid* (*Xerocomic Acid*) (XVIII) *and* 3′,4′,4-*Trihydroxypulvinic Acid* (*Isoxerocomic Acid*) (XIX).—A mixture of 3,4,4′-trimethoxypulvinic acid lactone (10 g), acetic acid (500 ml), and hydriodic acid (200 ml, *d* 1·7) was refluxed until almost all the lactone had dissolved (<0·5 h). The hot solution was filtered; the filtrate was evaporated to dryness under reduced pressure and the residual gum was dissolved in ether (250 ml). The solution was shaken with sodium thiosulphate solution (3 × 100 ml; 20%) to remove iodine and then with water. Evaporation of the dried ethereal solution gave a dark red gum which was dissolved in hot water (50 ml) and set aside. After 3 h the crystalline solid (2·8 g) was filtered off and recrystallised twice from water (50 ml) to give *isoxerocomic acid* (700 mg) as orange-red needles, m.p. 300—305° (Found: C, 60·8; H, 3·3. $C_{18}H_{12}O_8$ requires C, 60·7; H, 3·4%).

The aqueous mother liquor deposited a second crop of crystals (1·8 g) over 3 days consisting of a mixture of 3′,4′,4- and 3,4,4′-trihydroxypulvinic acids; these were

removed and the filtrate was evaporated to 5 ml and filtered hot. The red crystalline solid which was slowly deposited (900 mg) was recrystallised twice from water (5 ml) to yield *xerocomic acid* (510 mg) as red needles, m.p. 295° (decomp.) (Found: C, 60·4; H, 3·6%).

By heating the reaction mixture for 0·75 h the yield of crude 3′,4′,4-trihydroxypulvinic acid was reduced to 1·0 g and after 1 h the product was entirely 3,4,4′-trihydroxypulvinic acid (1·5 g).

Methylation of 3,4,4′-trihydroxypulvinic acid with diazomethane in ether gave methyl *O*-methyl-3,4,4′-trimethoxypulvinate, identical with that described previously. Similarly, 3′,4′,4-trihydroxypulvinic acid gave methyl *O*-methyl-3′,4′,4-trimethoxypulvinate.

Xerocomorubin (XXVII).—Hydrogen peroxide (0·4 ml; 20 vol.) and ammonium tungstate (5 mg) were added to 3′,4′,4-trihydroxypulvinic acid dissolved in a mixture of ethanol (1·25 ml), acetic acid (0·5 ml), and water (1·8 ml). A vigorous exothermic reaction took place. The mixture was set aside and the dark red crystalline solid which separated was recrystallised from acetic acid to yield *xerocomorubin* (89 mg) as red-brown needles, m.p. 285° (decomp.) (Found: C, 61·4; H, 2·8. $C_{18}H_{10}O_8$ requires C, 61·0; H, 2·8%); $\lambda_{max.}$ (EtOH) 257, 274·5, 287infl, 364infl, and 497 nm (log ε 4·14, 4·10, 4·03, 3·70, and 4·24).

Xerocomorubin Tetramethyl Ether.—Xerocomorubin (30 mg) was treated with an excess of ethereal diazomethane. After 1 h the excess of reagent was destroyed with acetic acid and the solvent was evaporated off. The residue crystallised from acetic acid or benzene to yield the *tetramethyl ether* (7 mg) as red needles, m.p. 213—216° (Found: C, 64·4; H, 4·45. $C_{22}H_{18}O_8$ requires C, 64·4; H, 4·4%); $\nu_{max.}$ (KBr) 1776 cm^{-1} (C=O, lactone); $\lambda_{max.}$ ($CHCl_3$) 272, 372, and 454 nm (log ε 3·88, 3·62, and 3·83).

Xerocomorubin Triacetate.—A mixture of xerocomorubin (30 mg), acetic anhydride (0·3 ml), and sulphuric acid (1 drop) was heated on a water-bath for 15 min. Cooling produced a yellow crystalline solid which was recrystallised from acetic acid to give the *triacetate* (18·8 mg) as yellow needles, m.p. 232—235° (Found: C, 60·2; H, 3·4. $C_{24}H_{16}O_{11}$ requires C, 60·0; H, 3·3%); $\nu_{max.}$ ($CHCl_3$) 3600—2500 (chelated OH), 1778 (acetate), and 1737 cm^{-1} (C=O, lactone); $\lambda_{max.}$ ($CHCl_3$) 342infl and 440 nm (log ε 3·70 and 4·19).

3,4,4′-*Tris(methoxymethoxy)pulvinic Acid Lactone* (VI).—Chloromethyl methyl ether (6 ml) was added dropwise to a stirred heated mixture of 3′,4′,4- and 3,4,4′-trihydroxypulvinic acids (3 g) and anhydrous potassium carbonate (10 g) in dry acetone (100 ml). After 1 h the mixture was filtered and the residue washed with acetone. Evaporation of the filtrate under reduced pressure yielded a red gum which was refluxed with acetic anhydride for 0·5 h. Cooling produced crystals of the *lactone* (VI) which, recrystallised from acetic acid, formed tiny yellow needles, m.p. 142—145° (Found: C, 61·5; H, 4·6. $C_{24}H_{22}O_{10}$ requires C, 61·3; H, 4·7%); $\nu_{max.}$ (KBr) 1825 and 1789 cm^{-1} (dilactone C=O).

Methanolysis of 3,4,4′-*Tris(methoxymethoxy)pulvinic Acid Lactone.*—A suspension of the lactone (1·2 g) in methanolic potassium hydroxide (100 ml; 2%) was stirred at room temperature (0·5 h), then poured into water (1 l). The solution was acidified with acetic acid and extracted with ether (3 × 100 ml). The extract was dried and evaporated to give an orange-yellow gum. Chromatography on a column of silica gel (40 × 4 cm) using solvent (2) gave two yellow bands. The first band gave an orange oil which crystallised slowly from methanol (0°) as orange needles. Recrystallisation from methanol or acetic acid gave *methyl* 3′,4′,4-*tris(methoxymethoxy)pulvinate* (XII) (292 mg) as yellow needles, m.p. 138—140° (Found: C, 59·8; H, 5·2. $C_{25}H_{26}O_{11}$ requires C, 59·75; H, 5·2%); $\nu_{max.}$ (KBr) 3440—2580 (OH), 1782 (C=O, lactone), and 1675 cm^{-1} (C=O, ester); τ ($CDCl_3$) 1·74 (1H), 1·84 (1H), 2·56—3·10 (5H), 4·66, 4·70, and 4·53 (each 2H), and 6·07, 6·41, 6·43, and 6·48 (each 3H).

The second band gave *methyl* 3-*hydroxy*-4,4′-*bis(methoxymethoxy)pulvinate* (VIII) (185 mg) as an orange oil (Found: C, 60·4; H, 5·2. $C_{23}H_{22}O_{10}$ requires C, 60·3; H, 5·0%); $\nu_{max.}$ ($CHCl_3$) 3540—2618 (OH), 1780 (C=O, lactone), and 1679 cm^{-1} (C=O, ester); τ ($CDCl_3$) 1·70—3·21 (8H), 4·71 and 4·77 (each 2H), and 6·13, 6·47, and 6·49 (each 3H).

Methyl 3,4,4′-*Trihydroxypulvinate* (*Methyl Xerocomate*) (IX).—A mixture of methyl 3-hydroxy-4,4′-bis(methoxymethoxy)pulvinate (100 mg), acetic acid (5 ml), and sulphuric acid (2 drops; 2N) was refluxed (3 min). The red solution was cooled, diluted with water, and extracted with ether. Evaporation of the ether and crystallisation of the residual gum from aqueous methanol (3×) gave *methyl xerocomate* (41 mg) as red rhombs, m.p. 258—261° (Found: C, 61·7; H, 3·75. $C_{19}H_{14}O_8$ requires C, 61·6; H, 3·8%); $\nu_{max.}$ (KBr) 3320—2550 (OH), 1740 (lactone C=O), and 1673 cm^{-1} (chelated ester C=O); $\lambda_{max.}$ (EtOH) 250, 270, 278infl, 340·5sh, and 397 nm (log ε 4·27, 4·27, 4·26, 4·04, and 4·14), $\lambda_{max.}$ (10% EtOH) 235, 253, 322, and 372infl nm (log ε 4·22, 4·23, 4·23, and 4·04), $\lambda_{max.}$ (10% EtOH + 2 drops 2N-NH_4OH) 246, 396, and 601 nm (log ε 4·25, 4·32, and 3·48); τ [$(CD_3)_2CO$] 1·33 (1H), 1·85 (1H), 2·16—3·08 (7H), and 6·06 (3H).

Treatment of the above ester with an excess of ethereal diazomethane gave methyl *O*-methyl-3,4,4′-trimethoxypulvinate, identical with that already described.

Similarly acidic hydrolysis of methyl 3′,4′,4-tris(methoxymethoxy)pulvinate gave *methyl isoxerocomate* (XIII) as lustrous orange leaflets (from aqueous methanol), m.p. 219—222° (Found: C, 61·2; H, 4·1%); $\nu_{max.}$ (KBr) 3400—2600 (OH), 1746 (lactone C=O), and 1671 cm^{-1} (chelated ester C=O); $\lambda_{max.}$ (EtOH) 248, 268, 283infl, 345infl, and 396 nm (log ε 4·25, 4·26, 4·17, 3·98, and 4·15), $\lambda_{max.}$ (10% EtOH) 234infl, 257, 310infl, 329, and 372infl nm (log ε 4·28, 4·33, 4·25, 4·30, and 4·03), $\lambda_{max.}$ (10% EtOH + 2 drops 2N-NH_4OH) 263 and 386 nm (log ε 4·37 and 4·28); τ [$(CD_3)_2CO$] 1·87 (1H), 1·96 (1H), 2·96—3·27 (5H), 6·05 (3H), and 6·83 (3H). Treatment of this with ethereal diazomethane gave methyl *O*-methyl-3′,4′,4-trimethoxypulvinate.

Methyl O-*Methyl*-3-*methoxy*-4,4′-*bis(methoxymethoxy)pulvinate.*—Methyl 3-hydroxy-4,4′-bis(methoxymethoxy)pulvinate (400 mg) was treated with an excess of ethereal diazomethane. After 3 h excess of reagent was destroyed with acetic acid and the solvent was removed under reduced pressure. The residual yellow oil was purified by p.l.c. using solvent (2); an intense yellow band (R_F 0·55) gave the *ether* (290 mg) as a lemon yellow oil (Found: C, 61·4; H, 5·4. $C_{25}H_{26}O_{10}$ requires C, 61·7; H, 5·35); $\nu_{max.}$ ($CHCl_3$) 1760 (lactone C=O) and 1724 cm^{-1} (ester C=O); τ ($CDCl_3$) 2·23—3·06 (7H), 4·70 and 4·76 (each 2H), 6·08 (6H), and 6·18, 6·46, and 6·50 (each 3H).

Methyl 3-*Acetoxy*-4,4′-*bis(methoxymethoxy)pulvinate.*—The hydroxy-ester (50 mg) in a mixture of acetic anhydride (3 ml) and pyridine (0·2 ml) was set aside at room temperature (24 h). The mixture was poured into water and extracted with ether. The extracts were dried and

evaporated and the residual yellow oil was purified by p.l.c. using solvent (2). A band at R_F 0·61 gave the *acetate* (32 mg) as a yellow oil (M^+ 500·133792. $C_{25}H_{24}O_{11}$ requires M, 500·131848); $\nu_{max.}$ ($CHCl_3$) 1770 (acetate C=O), 1761 (lactone C=O), and 1671 cm^{-1} (chelated ester C=O); τ ($CDCl_3$) 1·76—2·91 (7H), 4·72 (4H), and 6·06, 6·46, 6·48, and 7·65 (each 3H).

Methyl 3,4,4′-*Tris(methoxymethoxy)pulvinate* (X).—Chloromethyl methyl ether was added dropwise to a stirred refluxing suspension of methyl 3-hydroxy-4,4′-bis(methoxymethoxy)pulvinate (2 g) and anhydrous potassium carbonate (15 g) in dry acetone (150 ml). After 2 h the mixture was cooled, filtered, and evaporated under reduced pressure. The residue was purified by p.l.c. using solvent (2); elution of the R_F 0·49 band gave *methyl* O-(*methoxymethyl*)-3,4,4′-*tris(methoxymethoxy)pulvinate* as a pale yellow gum (M^+, 546. Calc. for $C_{27}H_{30}O_{12}$: M, 546); $\nu_{max.}$ ($CHCl_3$) 1764 (lactone C=O) and 1730 cm^{-1} (ester C=O). Elution of the R_F 0·59 band gave unchanged starting material. The R_F 0·61 band gave an orange oil which with diazomethane in ether gave an oily product. Crystallisation of this from alcohol (0°) gave *methyl* O-*methyl*-3,4,4′-*tris(methoxymethoxy)pulvinate* (XV) (47 mg) as pale yellow rosettes, m.p. 106—109° (Found: C, 60·5; H, 5·4. $C_{26}H_{28}O_{11}$ requires C, 60·5; H, 5·4%); $\nu_{max.}$ (KBr) 1761 (lactone C=O) and 1720 cm^{-1} (ester C=O).

Similarly, methylation of methyl 3′,4′,4-tris(methoxymethoxy)pulvinate (140 mg) gave *methyl* O-*methyl*-3′,4′,4-*tris(methoxymethoxy)pulvinate* (XVII) (75 mg) as pale yellow needles (from ethanol at 0°), m.p. 91—93° (Found: C, 60·4; H, 5·4%); $\nu_{max.}$ (KBr) 1764 (lactone C=O) and 1723 cm^{-1} (ester C=O).

5-[3,4-*Bis(methoxymethoxy)phenyl*]-4-*methoxy*-2-(4-*methoxymethoxyphenyl)cyclopent*-4-*ene*-1,3-*dione* (XXI).—Methyl *O*-methyl-3,4,4′-tris(methoxymethoxy)pulvinate (90 mg) was rearranged with methanolic potassium hydroxide (4%) as described for the methoxy-analogue. Crystallisation from alcohol gave the *dione* (38 mg) as yellow needles, m.p. 95° (Found: C, 63·1; H, 6·0. $C_{24}H_{26}O_9$ requires C, 62·9; H, 5·7%); $\nu_{max.}$ (KBr) 1726, 1687, and 1671 cm^{-1}; $\lambda_{max.}$ (EtOH) 225sh, 249, 269infl, and 355 nm (log ε 4·19, 4·12, 3·98, and 3·80); τ ($CDCl_3$) 1·97—2·95 (7H), 4·65, 4·67, and 4·78 (each 2H), 5·53 (3H), 5·93 (1H), and 6·42, 6·44, and 6·50 (each 3H). A similar rearrangement of methyl *O*-methyl-3′,4′,4-tris(methoxymethoxy)pulvinate (347 mg) gave 2-(3,4-*bismethoxymethoxyphenyl*)-4-*methoxy*-5-(4-*methoxymethoxyphenyl)cyclopent*-4-*ene*-1,3-*dione* (XXIII) (149 mg) as pale yellow needles (from ethanol), m.p. 62—64° (Found: C, 62·7; H, 5·7%); $\nu_{max.}$ (KBr) 1733 and 1686 cm^{-1}; $\lambda_{max.}$ (EtOH) 230, 248, and 350 nm (log ε 4·24, 4·23, and 3·95); τ ($CDCl_3$) 1·83 (1H), 1·92 (1H), 2·72—3·21 (5H), 4·69 (4H), 4·72 (2H), 5·52 (3H), 5·93 (1H), 6·44 (3H), and 6·46 (6H).

Involutin, (±)-cis-5-(3,4-*Dihydroxyphenyl*)-3,4-*dihydroxy*-2-(4-*hydroxyphenyl)cyclopent*-2-*enone* (III).—2-(3,4-Bismethoxymethoxyphenyl)-4-methoxy-5-(4-methoxymethoxyphenyl)cyclopent-4-ene-1,3-dione (300 mg) in ethanol (15 ml) was added dropwise with stirring to a solution of sodium borohydride (150 mg) in water (9 ml) at room temperature. The yellow colour of the solution was discharged over 0·5 h. The mixture was diluted with water (50 ml), acidified with hydrochloric acid (2N), and set aside overnight. It was then extracted with ether (3 × 30 ml); the extracts were dried and evaporated. The residue was refluxed for 1 min with acetic acid (1 ml) and sulphuric acid (1 drop; 2N). The solution, which darkened during the hydrolysis, was cooled and diluted with water (10 ml); the amorphous grey solid which separated was filtered off. The filtrate was extracted with ethyl acetate (3 × 10 ml) and the extract was dried and evaporated. The residue was examined by t.l.c. on cellulose plates in ethyl acetate–formic acid (99 : 1). Three components were present (R_F 0·97, 0·82, and 0·65) (blue spots with $FeCl_3$); the spot R_F 0·65 corresponded to the spot from an authentic sample of involution.

A similar reduction and hydrolysis of 5-(3,4-bismethoxymethoxyphenyl)-4-methoxy-2-(4-methoxymethoxyphenyl)-cyclopent-4-ene-1,3-dione gave a product which on t.l.c. cellulose plates ran with the solvent front.

Xerocomic Acid from Gomphidius Rutilus.—An aqueous solution of the extracts of *G. rutilus* [1] was continuously extracted with ether (500 ml) and the extract was evaporated to a red-brown gum. The gum was purified by p.l.c. using solvent (1). The major yellow band (R_F 0·54) gave a red solid, one half of which was crystallised from formic acid to give xerocomic acid (54 mg) as red needles, m.p. 295° (decomp.) (Found: C, 60·70; H, 3·5%), which was identical with synthetic 3,4,4′-trihydroxypulvinic acid. The remaining chromatographically pure solid was treated with an excess of ethereal diazomethane. Crystallisation of the product from ethanol (2×) gave methyl *O*-methyl-3,4,4′-trimethoxypulvinate (42 mg), m.p. 148—149° (Found: C, 65·1; H, 5·2%), identical with the compound already described.

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