Metabolites of Proteaceae. Part V.† Reflexin and Conocarpic Acid from *Leucospermum reflexum* Buek *ex* Meisner, and the Phenol–Dienone Rearrangement of Reflexin and Conocarpin

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Reflexin is obtained from leaves of *Leucospermum reflexum* Buek *ex* Meisner by extraction with methanol and is shown to correspond to the methyl ester of the ring-A-opened form of conocarpin, *viz.* conocarpic acid. Both reflexin and conocarpin undergo phenol–dienone conversion into a dibromo-spiro-cyclohexadienone cyclic ether system on treatment with bromine in water. A model system, 3-(4-hydroxyphenyl)propan-1-ol, reacts in the same manner.

LEAVES of *Leucospermum reflexum* Buek *ex* Meisner (family Proteacea) on extraction with methanol yielded a number of phenolic compounds of which six (LR1—6, in order of increasing polarity) could be separated by paper chromatography, compounds LR2, LR3 and LR5 occurring as major constituents. Constituent LR2 was shown to be conocarpin, $C_{15}H_{16}O_8$, previously [1] obtained from *Leucospermum conocarpodendron* (L) Buek., and LR5 (not obtained in crystalline form) was found to yield conocarpin on heating. Compound LR3 (reflexin), a stable crystalline product, could also be converted into conocarpin; thus these three compounds are closely related.

Reflexin, $C_{16}H_{20}O_9$, m.p. 110—114°, $[\alpha]_D$ +36° (50% ethanol), is a *para*-substituted ($\nu_{max.}$ 820 cm^{-1}) phenol containing an ester ($\nu_{max.}$ 1733 cm^{-1}) and a γ-lactone group ($\nu_{max.}$ 1765 cm^{-1}), as well as four alcoholic hydroxy-groups, altogether five hydroxy-groups being shown by deuterium exchange and the formation of crystalline penta-acetate and pentabenzoate. Hydrolysis followed by relactonisation converted reflexin into conocarpin (I), so that reflexin could have structure (II) or (IV).

Structure (II), rather than (IV), was indicated by quantitative periodic acid titrations (acid-start and alkaline-start procedures [2]). The mass spectral fragmentation pattern was essentially identical with those of conocarpin and leucodrin; [1,3] no molecular ion was, however, observed at *m/e* 356, thus suggesting the ready loss of methanol, by ring closure on to the hydroxy-group γ to the ester group, with formation of conocarpin in the ion source.

Such an elimination of methanol, as against loss of methoxy-radical to give the species (M − 31), is known [4]

† Previous papers in this series are assigned the following Part numbers: Part I, G. W. Perold and K. G. R. Pachler, *J. Chem. Soc.* (*C*), 1966, 1918; Part II, G. W. Perold and H. K. L. Hundt, *ibid.*, 1966, 1924; Part III, P. E. J. Kruger and G. W. Perold, *ibid.*, 1970, 2127; Part IV, G. W. Perold, A. S. Howard, and H. K. L. Hundt, *ibid.*, 1971, 3136; see also G. W. Perold and H. K. L. Hundt, *Chem. Comm.*, 1970, 712.

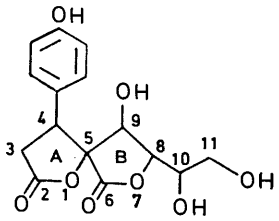
[1] P. E. J. Kruger and G. W. Perold, *J. Chem. Soc.* (*C*), 1970, 2127.

[2] G. W. Perold and K. G. R. Pachler, *J. Chem. Soc.* (*C*), 1966, 1923.

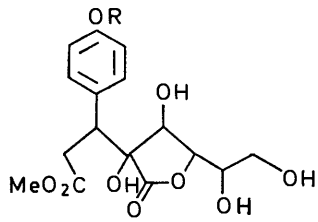
[3] P. E. J. Kruger, Ph.D. Thesis, University of the Witwatersrand, Johannesburg, 1968.

[4] F. W. McLafferty and R. S. Gohlke, *Analyt. Chem.*, 1959, **31**, 2076.

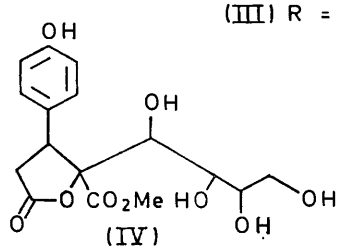
even for methyl esters containing only a hydrogen atom in the γ-position, and would here occur much more easily. Similarly the absence of a peak for $(M - 59)$ in the mass spectrum of reflexin, expected for the



(I)



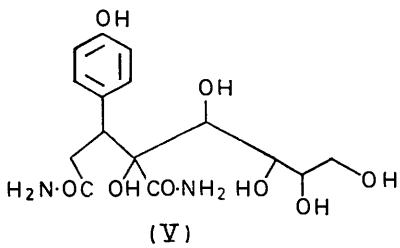
(II) R = H
(III) R = Me



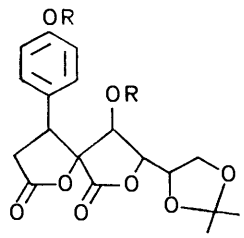
(IV)

alternative bond cleavage α to the ester carbonyl group, supports the primary formation of conocarpin from reflexin in the ion source. On the other hand, reflexin penta-acetate does in its mass spectrum display a weak molecular ion peak, at *m/e* 566, in accord with this proposal.

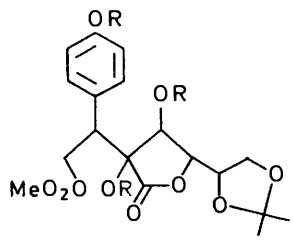
The interrelationship of reflexin and conocarpin was indicated by converting both of them into the same dihydroxy-diamide (V), and into the same (conocarpin) 10,11-dimethyl acetal (VI) when this was prepared under conditions of acid catalysis. The difference between the two compounds was demonstrated by the formation



(V)



(VI) R = H
(VII) R = Ac

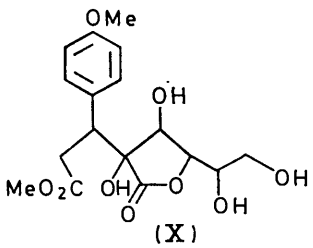


(VIII) R = H
(IX) R = Ac

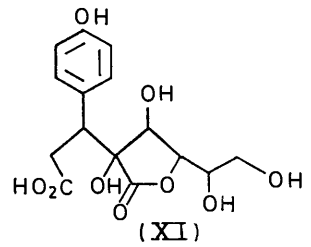
of a different 10,11-dimethyl acetal (VIII) from reflexin under neutral conditions. These and other derivatives are described in the Experimental section.

Structure (II) for reflexin, as well as its relationship to conocarpin, was demonstrated by the formation from both of the same methyl ether methyl ester (X), which arises [1] from conocarpin (I) by alcoholytic opening of lactone ring A together with etherification

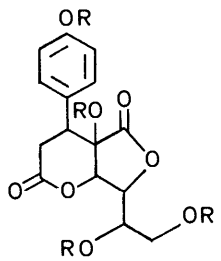
This close relation, together with the observed formation of conocarpin on heating the more polar plant constituent LR5, makes it likely that reflexin is an artefact arising (indirectly) from LR5 [for which the ring-A-opened hydroxy-acid structure (XI) may be considered] during the extraction of the plant material with methanol. This was supported by carrying out an extraction of the leaves with water; phenols LR5 and LR2 (conocarpin) were shown to occur in the extract, but no reflexin (see Experimental section). As the metabolite LR5 was not obtained in pure crystalline form, it is proposed to retain the designation reflexin for phenol LR3, as this is the readily available and



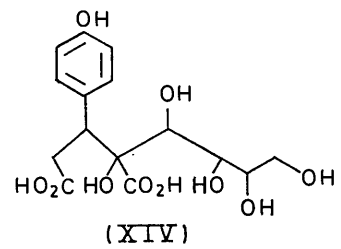
(X)



(XI)



(XII) R = H
(XIII) R = Ac



(XIV)

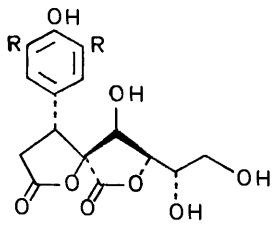
immediate derivative of the native phenolic hydroxy-acid LR5 (XI).

This relationship was supported by the isolation from an ethanolic extract of the leaves of a crystalline lead salt (see Experimental section) of a phenolic hydroxy-acid, $C_{15}H_{20}O_{10}$, which on ring closure yielded (in relatively low yield) a crystalline monolactonic conocarpic acid which we formulate as (XI), m.p. 126—136°, showing carbonyl absorption at 1715 (CO_2H) and 1785 cm^{-1} (γ-lactone). Its mass spectrum was identical with that of conocarpin, so that ready closure to a dilactone is indicated. Its stereochemical relation to conocarpin was supported by its methylation and periodate oxidation to yield (+)-*p*-methoxyphenylsuccinic acid.[1] On acetylation in pyridine it however yielded a crystalline *allo*-conocarpin tetra-acetate, m.p. 190—191°, for which the δγ-dilactone structure (XIII) was established by its i.r. and n.m.r. characteristics (see Experimental section). The same *allo*-conocarpin tetra-acetate could then be obtained starting from pure conocarpin by opening both lactone rings with

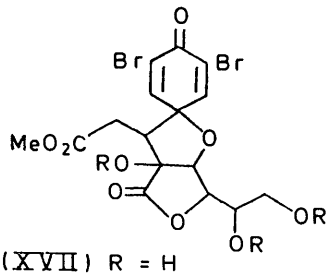
2 mol. equiv. of alkali and then acetylating as before; under these conditions both the *allo*- and the normal conocarpin tetra-acetates were formed (see Experimental section). It therefore appears that the fully open polyhydroxy-dicarboxylic acid (XIV) in the conocarpin series can lactonise to the δγ- (XII) or the γγ-dilactone (I), the lesser steric hindrance between the aromatic ring and lactone ring B for (XII) as against (I) making the δγ-lactone closure an alternative. This would at the same time explain the ready formation of reflexin by methanolysis of the dilactone (XII) as an intermediate, rather than by direct esterification of the free acid (XI). A paper chromatographic study of conocarpic acid confirmed (see Experimental section) the ease with which it reacts with methanol; conocarpin does not react in this manner.

Bromination of reflexin in aqueous solution led to a novel phenol–dienone rearrangement which proved to be of use in elucidating its stereochemistry (see following paper).

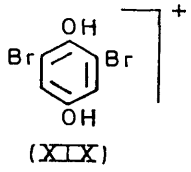
The related compound leucodrin (XV) on bromination yields [6] the expected 2,6-dibromophenol derivative (XVI), a stable compound whose detailed structure was fully assigned on the basis of an *X*-ray diffraction



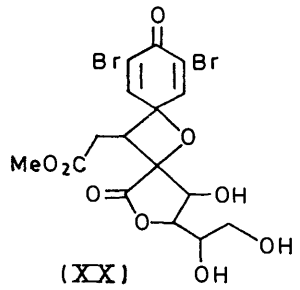
(XV) R = H
(XVI) R = Br



(XVII) R = H
(XVIII) R = Ac



(XIX)

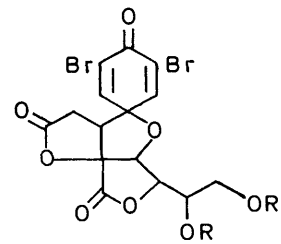


(XX)

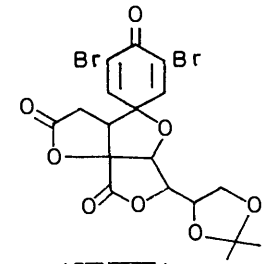
analysis.[7] The same reaction in the case of reflexin ($C_{16}H_{20}O_9$) now yielded a dibromo-compound, $C_{16}H_{16}Br_2O_9$, so that an additional two hydrogen atoms had been lost in this transformation. This product, m.p. 143—145°, was found to be the dibromoquinolide (XVII). It showed no aromatic ring vibrations in the i.r. and the cyclohexa-2,5-dienone system was demonstrated by its i.r. and u.v. characteristics (see Experimental section). Its mass spectrum confirmed its composition (weak parent peak as the expected triplet at *m/e* 510, 512, 514) and furthermore showed the presence in its structure of a new bond between oxygen and the carbocyclic ring of reflexin; the base peak, a triplet at *m/e* 266, 268, 270, was shown to be due to an ion of the composition $C_6H_4Br_2O_2$ by accurate mass determination. This species is formulated as (XIX). The full fragmentation pattern of this quinolide is in accord with structure (XVII) and has been fully analysed.[8] The n.m.r. spectrum of the quinolide (100 MHz; C_5D_5N) was clearly resolved: the two olefinic protons resonate as doublets at δ 7·7 and 8·1 p.p.m. (separation for each 2·5 Hz),[9] their non-equivalence supporting the rigid spiro-bonding of the cyclic ether to the cyclohexadienone system. The remaining resonances are in accord with structure (XVII) (see Experimental section).

Acetylation of this bromoquinolide (by various procedures) gave a triacetate, $C_{22}H_{22}Br_2O_{12}$ (XVIII) (as a glass), which furthermore afforded evidence that the hydroxy-group involved in the formation of the quinolide was that at the 9-position (to give the five-membered cyclic ether shown) and not the 5-substituent [which would give rise to a four-membered spiro-cyclic ether (XX)]. Thus on acetylation of the dibromoreflexin quinolide (XVII), the n.m.r. signals of H-10 and of H-11 shifted downfield in the expected [5] manner, while the resonance of H-9, clearly identifiable as showing the only simple one-proton doublet [separation 4 Hz for (XVII), 5 Hz for (XVIII)] in this region, remained unchanged at δ 5·0 p.p.m. when the two compounds were directly compared in hexadeuterioacetone solution.

In view of the close similarity of reflexin and conocarpin, the bromination of conocarpin was also studied and found to afford the precisely analogous dibromoconocarpin quinolide (XXI). All its data are in accord with this structure and are in the Experimental section, as are also the data for the derived 10,11-dimethyl acetal (XXIII).



(XXI) R = H
(XXII) R = Ac



(XXIII)

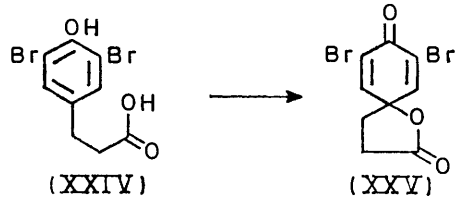
That this phenol–dienone change, involving amongst other things the loss of the hydrogen atom of the phenolic hydroxy-group, occurs with great ease, was furthermore demonstrated by the ready formation of dibromoconocarpin quinolide (XXI) from cono-

[5] L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' 2nd edn., Pergamon, Oxford, 1969, p. 164.
[6] W. S. Rapson, *J. Chem. Soc.*, 1938, 286.
[7] R. D. Diamand and D. Rogers, *Proc. Chem. Soc.*, 1964, 63.
[8] A. J. Hodgkinson, Ph.D. Thesis, University of the Witwatersrand, Johannesburg, 1970.
R. Barner, *Helv. Chim. Acta*, 1965, **48**, 94.

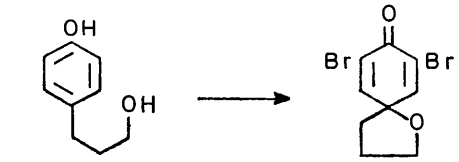
carpin methyl ether [*cf.* structure (I)]: the aryl methoxy-function is thus readily demethylated in this process.

The phenol–dienone change so encountered for reflexin and conocarpin was subsequently * found to be similar to the reaction studied in connection with tyrosine derivatives by Corey [10] and by Witkop,[11] using 3,5-dibromophloretic acid (XXIV) as a model system. In their work, conversion of the aromatic into the quinolide system (XXV) resulted from internal nucleophilic attack by an oxycarbonyl group with the formation of a spirodienone–lactone system and this type of reaction is reported [10] to be prevented by prior methylation of the phenolic hydroxy-group. The reaction now reported is a striking example of the role of a simple alcoholic hydroxy-group as the nucleophile in this transformation, with formation of a spirodienone cyclic ether as the product; prior methylation of the phenolic hydroxy-group does not prevent the reaction from proceeding smoothly.

We have also studied a model system, 3-(4-hydroxyphenyl)propan-1-ol (XXVI), related directly to the reflexin and conocarpin structures. Treatment of

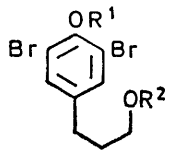


(XXIV) (XXV)



(XXVI) (XXVII)

the model compound (XXVI) with bromine in aqueous ethyl acetate † solution readily afforded the expected dibromoquinolide (XXVII), *i.e.* 7,9-dibromo-1-oxaspiro-[4,5]deca-6,9-dien-8-one, m.p. 119—120°, whose spectral data (see Experimental section) are in full accord with



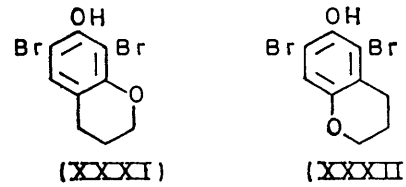
(XXVIII) $R^1 = R^2 = H$
(XXIX) $R^1 = H, R^2 = Ac$
(XXX) $R^1 = R^2 = Ac$

this structure and correspond with the data for its analogues obtained from reflexin and conocarpin. In other solvents the reaction proceeded otherwise: in methanol, the product was the dibromophenol (XXVIII), and in acetic acid solution it was 3-(3,5-dibromo-4-hydroxyphenyl)propyl acetate (XXIX) (details in Experimental section).

* We thank Professor A. Eschenmoser, ETH, Zürich, for drawing our attention to this analogy at a later stage.

† Ethyl acetate was here needed to obtain a homogeneous solution of the less polar model compound.

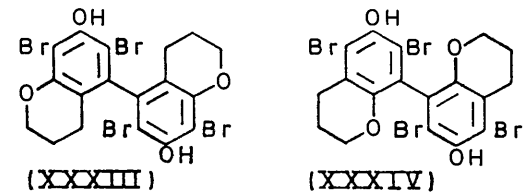
The further dienone–phenol reaction of the quinolide (XXI) obtained from conocarpin proved useful in discussing its stereochemistry (see following paper), and the model quinolide (XXVII) was therefore studied in this regard as well. It underwent a dienone–phenol change in the presence of boron trifluoride–ether complex or aluminium chloride to form a dibromohydroxychroman, *i.e.* (XXXI) or (XXXII). A firm decision between the two possibilities has not yet been obtained. Substituent constants for expected chemical shifts are



(XXXI) (XXXII)

uncertain on account of the numerous bulky substituents here involved.[12,13]

The action of cold concentrated sulphuric acid on the model dibromoquinolide (XXVII) afforded a diphenol, $C_{18}H_{14}Br_4O_4$, containing no aromatic protons. This compound is therefore another product resulting from a dienone–phenol rearrangememt of the quinolide; its formation suggests an unusually ready aryl-to-biaryl condensation and it is therefore formulated as the bichroman (XXXIII) or (XXXIV).



(XXXIII) (XXXIV)

EXPERIMENTAL

M.p.s were taken on a Kofler micro hot-stage apparatus. Spectra were obtained on Beckman DB-G grating (u.v.), Perkin-Elmer 521 (i.r.), A.E.I. MS 9 and Varian-MAT CH5 (mass), and Varian HA100 and Hitachi-Perkin-Elmer R20 (n.m.r.) spectrometers. I.r. spectra were taken for potassium bromide dispersions. Optical rotations were determined for solutions in 96% ethanol on a Zeiss-Winkel model 166905 polarimeter. N.m.r. data are expressed in p.p.m. relative to tetramethylsilane as internal standard; splitting patterns are quoted in the form AB, *etc.* and separations (*S* in Hz) are read from the spectra. T.l.c. was performed on plates of silica gel GF_{254} (nach Stahl). All column chromatography was performed over Merck silica gel (0·05—0·20 mm) which was heated to 230° before use. Paper chromatograms (p.c.s) were run in butanol–toluene (1 : 1) as before [14] on paper impregnated with glycerol. Reactions were carried out at room temperature unless specified otherwise.

Air-dried leaves of *Leucospermum reflexum* Buek *ex*

[10] E. J. Corey and L. F. Haefele, *J. Amer. Chem. Soc.*, 1959, **81**, 2225.

[11] G. L. Schmir, L. A. Cohen, and B. Witkop, *J. Amer. Chem. Soc.*, 1959, **81**, 2228.

[12] N. van Meurs, *Rec. Trav. chim.*, 1968, **87**, 147.

[13] P. Diehl, *Helv. Chim. Acta*, 1961, **44**, 829.

[14] Ref. 1, p. 2131.

Meisner were milled to a powder. This (0·5 g) was extracted with methanol: a p.c. on the extract sprayed with Pauly's reagent showed six phenolic spots at R_F 0·00, 0·16, 0·28, 0·47, 0·59, and 0·80. On immersion of a similar p.c. in alkaline phenolphthalein solution [14] the spots for LR2, LR3, and LR5 at R_F 0·59, 0·47, and 0·16 (the major constituents) showed as white spots on a pink background. T.l.c. of the extract in benzene–butanone (1 : 3 v/v) showed these constituents at R_F 0·60, 0·52, and 0·20, respectively. The extract was dried to a brown foam (0·1 g) which was heated at 140° for 10 min; the residue was dissolved in 50% ethanol (2 ml) and run on t.l.c. as before; the spot for LR5 had disappeared, and the intensity of the spot for LR2 had increased.

Extraction of the milled leaf material (0·12 g) with water (2 ml) for 24 h gave an extract which on t.l.c. showed (Pauly reagent) main spots only for LR2 and LR5 (and no LR3).

Reflexin and Conocarpin.—The leaf powder (1 kg) was extracted (Soxhlet) with methanol to afford 270 g of dried extract. This was spread on silica gel (300 g) and chromatographed over silica gel (1 kg), the elution with benzene–butanone (1 : 3 v/v) being followed by t.l.c. Fractions containing mainly LR2 and LR3 were combined and dried (62 g) and rechromatographed twice as before. Fractions showing only a spot for LR2 were combined and dried to a colourless foam (29 g) which from butanone–benzene mixtures gave conocarpin (20·6 g), m.p. 184·5—186°, identical with conocarpin obtained [1] earlier. Fractions containing essentially only LR3 were combined and finally chromatographed in benzene–butanone (1 : 1 v/v). The crude LR3 (10·4 g) from benzene–butanone mixtures gave *reflexin* (9·6 g), m.p. 110—114°. The use of basic lead acetate [14] led to a lower yield of reflexin. The crystals tended to retain butanone and were obtained solvent-free only from boiling n-propyl acetate as needles, m.p. 110—114° [Found: C, 53·8, 53·8; H, 5·9, 5·7%; equiv. wt. (by saponification), 174. $C_{16}H_{20}O_9$ requires C, 53·9; H, 5·6%; equiv. wt. (lactone + ester), 178], $[\alpha]_D$ +36° (*c* 0·95), $\lambda_{max.}$ (50% EtOH) 227 and 277 nm (ε 8600 and 1540), $\lambda_{max.}$ (0·1N-NaOH in 50% EtOH) 245 and 296 nm (ε 8640 and 5690); $\nu_{max.}$ 3409 and 3110 (OH), 1765 and 1733 (C=O), and 820 cm^{-1} (1,4-substituted benzene ring), *m/e* 324, 220, 165, 147, 120 (100%), and 119; δ [100 MHz; $(CD_3)_2CO$] 7·3 and 6·7 (A_2B_2, *S* 9, arom.), 4·8 (A of AB, *S* 5·5, lactone ring), 8·1, 5·3, and 3·0 (1H each, OH), 3·45 (A_3, OCH_3) (as the only clearly assignable resonances *). Periodic acid titrations showed (consumption of mol. equiv. of HIO_4) for acid-start,[2] 2·1, 2·3, 2·1, and 3·3 after 1, 2, 5, and 19 h; for alkaline-start,[2] 4·1, 4·2, 4·4, and 4·8 after 1, 2, 5, and 19 h.

Reflexin penta-acetate was obtained by keeping reflexin (300 mg) in pyridine (3 ml) and acetic anhydride (6 ml) for 16 h and distilled at 240° and 0·1 Torr as a glass (330 mg) which showed only one spot on t.l.c. [R_F 0·42 in benzene–butanone (1 : 6 v/v)] and from acetic acid–water gave crystals (200 mg), m.p. 118—119° (Found: C, 55·2; H, 5·3. $C_{26}H_{30}O_{14}$ requires C, 55·1; H, 5·4%), *m/e* 566 (M^+, weak), 465, 464, 433, 422, 302, 221, 179 (100%), 137, and 120; δ (60 MHz; $CDCl_3$) 7·4 and 7·0 (A_2B_2, *S* 9, arom.), 3·8, 3·2, and 2·7 (ABX, *S* 9, 4, and 16, ester side-chain), 6·1 and 4·3 (AB, *S* 9, lactone ring), 5·2 and 4·2 (A_2B, *S* 6·5, glycol side-chain), 2·27, 2·21, 2·12, 2·05, and 1·64 (5 × A_3, OAc), and 3·54 (A_3, OCH_3). *Reflexin pentabenzoate* was obtained from reflexin (300 mg) in pyridine (5 ml) and benzoyl chloride (2 ml) after 16 h and chromatographed in benzene–ethyl acetate over silica gel (500 mg crude product); from aqueous methanol it gave crystals (352 mg), m.p. 70—73° (Found: C, 69·8; H, 4·5. $C_{51}H_{40}O_{14}$ requires C, 69·9; H, 4·6%), δ (60 MHz; $CDCl_3$) 7·1 (A_2 of A_2B_2, *S* 8·5, arom.; the other part of the A_2B_2 pattern is obscured by the benzoate protons), *ca.* 7·5 and *ca.* 8·1 (25H, m, benzoate protons), 4·2, 3·4, and 3·0 (ABX, *S* 10, 4, and 16, ester side-chain), 6·7 and *ca.* 4·7 (AB, *S* 8, lactone ring), 5·8 and *ca.* 4·7 (A_2B), and 3·56 (A, OCH_3).

Conocarpin from Reflexin.—Reflexin (356 mg) was refluxed in N-sodium hydroxide (20 ml) for 20 min and the solution was then distilled (2 ml of distillate) on to 3,5-dinitrobenzoyl chloride (290 mg). This mixture was warmed at 95° for 2 min and treated with saturated aqueous sodium hydrogen carbonate solution (2 ml) to give [15] methyl 3,5-dinitrobenzoate (160 mg), m.p. and mixed m.p. 106—107° (lit., 109°). The aqueous distilland was acidified with hydrochloric acid, kept for 10 h, and extracted with ether, and the recovered product was chromatographed to give conocarpin (200 mg), m.p. 178—180° (from benzene–butanone) (Found: C, 55·6; H, 4·9. $C_{15}H_{16}O_8$ requires C, 55·6; H, 5·0%), $[\alpha]_D$ +76° (*c* 0·90), $\nu_{max.}$ 3440, 3300, and 3140 (OH), and 1785 cm^{-1} (C=O) as before.

The Dihydroxy-diamide (V).—(*a*) Conocarpin (200 mg) was kept in absolute methanol (20 ml) saturated with ammonia gas for 24 h; the solution was evaporated and the residue crystallised from methanol–ethyl acetate to give *compound* (V) (150 mg), crystals, m.p. 167—168° (Found: C, 50·2; H, 6·2; N, 7·8. $C_{15}H_{22}N_2O_8$ requires C, 50·3; H, 6·1; N, 7·8%), $\nu_{max.}$ 3330 (OH) and 1660 cm^{-1} (C=O).

(*b*) Reflexin (500 mg), treated similarly, gave compound (V) (300 mg), m.p. and mixed m.p. 166—168° (Found: C, 50·4; H, 6·3; N, 7·8%), identical (i.r. spectra) with the previous sample

Conocarpin Dimethyl Acetal (VI).—(*a*) Conocarpin (200 mg) was kept for 16 h in dry acetone (30 ml) containing hydrogen chloride gas (0·15 g). Excess of lead carbonate was added, the mixture was filtered and the filtrate was evaporated; the residue crystallised from acetone–benzene to give the *acetal* (VI) (120 mg), m.p. 185—187° (Found: C, 59·4; H, 5·6. $C_{18}H_{20}O_8$ requires C, 59·3; H, 5·5%), $[\alpha]_D$ +55° (*c* 1·41), $\nu_{max.}$ 3430 and 3320 (OH) and 1810 and 1785 cm^{-1} (C=O). Hydrolysis with dilute hydrochloric acid afforded conocarpin.

(*b*) Reflexin (500 mg), similarly treated, gave the acetal (VI) (280 mg), m.p. 185—187° (Found: C, 59·3; H, 5·5%), $[\alpha]_D$ +53° (*c* 1·30) $\nu_{max.}$ as before, *m/e* 364 (M^+, weak), 363, 348, 147, 120 (100%), and 101. A *diacetate* (VII), prepared in pyridine (1·5 ml) and acetic anhydride (3 ml), was obtained as a distilled glass (110 mg; at 250° and 0·1 Torr) from (VI) (150 mg) (Found: C, 58·7; H, 5·4. $C_{22}H_{24}O_{10}$ requires C, 58·9; H, 5·4%), *m/e* 448 (M^+, weak), 434, 433, 391, 120, 101, and 43 (100%).

Reflexin Dimethyl Acetal (VIII).—Reflexin (500 mg) was kept in dry acetone (200 ml) over anhydrous copper sulphate (5 g) for 16 h. The mixture was filtered and evaporated; the residue (550 mg) afforded *crystals* (310 mg) (from acetone), m.p. 148—150° (Found: C, 57·7; H, 6·3. $C_{19}H_{24}O_9$ requires C, 57·6; H, 6·1%), $\nu_{max.}$ 3445

* In all n.m.r. spectra of this series H-8 is also coupled with H-10 in the side-chain (*S ca.* 2—4).

[15] H. Henstock, *J. Chem. Soc.*, 1933, 216; W. A. Lipscomb and R. H. Baker, *J. Amer. Chem. Soc.*, 1942, **64**, 179.

and 3390 (OH) and 1775 and 1745 cm^{-1} (C=O). The *triacetate* (IX) was obtained from the acetal (100 mg) in pyridine and acetic anhydride as before and crystallised (yield 90 mg) from acetone with m.p. 182—184° (Found: C, 57·5; H, 5·8. $C_{25}H_{30}O_{12}$ requires C, 57·5; H, 5·8%), δ (60 MHz; $CDCl_3$) 7·4 and 7·0 (A_2B_2, *S* 9, arom.), 3·8, 3·2, and 2·7 (ABX, *S* 9·5, 4, and 16·5, ester side-chain), 6·2 and 4·2 (AB, *S* 8, lactone ring), 2·27, 2·21, and 1·64 (3 A_3, acetate), 3·52 (A_3, OCH_3), and 1·40 and 1·33 (2 A_3, *gem*-dimethyl).

Conocarpic Acid.—Milled leaves (1·18 kg) of *L. reflexum* were twice steeped for 20 h in 96% ethanol (5 l); the combined extracts were concentrated *in vacuo* at *ca.* 30° to a volume of 3 l and treated with saturated basic lead acetate solution (600 ml); the initial precipitate was filtered off and washed with water and the filtrate (9 l) was concentrated to a syrup (200 ml) which deposited lead salts (68·5 g). This salt mixture was extracted with boiling water (500 ml) to leave a less soluble salt (33·5 g) which crystallised from large volumes of hot water in fine *needles* (Found: C, 30·8. 31·2; H, 3·5, 3·5; Pb, 35·4, 35·6. $C_{15}H_{18}O_{10}$-Pb,H_2O requires C, 30·9; H, 3·5; Pb, 35·5%). This salt (25·6 g) was suspended in water (300 ml) and treated with hydrogen sulphide gas in excess; the filtrate from the lead sulphide was evaporated, finally for 1 h at 140°, to a glass (13·5 g) [Found for a similar sample: C, 52·6, 52·2; H, 5·3, 5·5%; equiv. wt. (by direct titration) 359, 361. $C_{15}H_{18}O_9$ requires C, 52·6; H, 5·3%; equiv. wt. (monocarboxylic acid), 342; a 3% solution of it in water had pH *ca.* 3·5]. It crystallised from water in fine needles (2·2 g) of *conocarpic acid*, m.p. 126—136° (Found: C, 52·5; H, 5·5. $C_{15}H_{18}O_9$ requires C, 52·6; H, 5·3%), $[\alpha]_D$ −117° (*c* 0·95), $\lambda_{max.}$ (MeOH) 228 and 277 nm (ε 9700 and 1630), $\lambda_{max.}$ (0·1N-NaOH in MeOH) 243 and 292 nm (ε 9100 and 1830), $\nu_{max.}$ 3530, 3470, 3370, and 3210 (OH), and 1785 and 1715 cm^{-1} (C=O), *m/e* 324 (*M* − H_2O), 220, 147, 121, 120 (100%), 119, 91, and 43, δ [$(CD_3)_2SO$] 6·9 and 6·7 (A_2B_2, arom.), 3·1 and 2·8 (AB, *S* 17, of ABX, lactone A), 5·1 and 4·0 (AB, *S* 7, of lactone B), 3·6—3·1 (m, A_2B, side-chain), and 9·4, 5·4, and 4·7 (A, OH); periodic acid consumption (mol. equiv./h elapsed): acid-start, 1·07/1, 1·01/2, 1·16/5, and 1·24/19, alkaline-start, 3·92/1, 3·97/2, 3·88/5, and 4·15/19. On a paper chromatogram it ran as a streak when applied to the baseline in aqueous solution, but when applied in methanolic solution it ran exactly as did reflexin as a single spot at R_F 0·47; when its methanolic spot (100 μg) on the base-line was treated with 2 μl of 10M-hydrochloric acid and again dried before running the p.c., it again behaved as did reflexin itself, showing four spots at R_F 0·04, 0·13 (this spot giving an acidic reaction with Bromocresol Green indicator, *i.e.* for pH < 4), 0·47 (reflexin), and 0·57 (conocarpin).

allo-*Conocarpin Tetra-acetate* (XIII).—(a) *From conocarpic acid.* Conocarpic acid (300 mg) kept in pyridine (3 ml) and acetic anhydride (6 ml) for 16 h gave allo-*conocarpin tetra-acetate*, which crystallised (yield 300 mg) from acetic acid, m.p. 190—191° (Found: C, 56·0; H, 5·0. $C_{23}H_{24}O_{12}$ requires C, 56·1; H, 4·9%), $\nu_{max.}$ 1810 and 1775 cm^{-1} (C=O), *m/e* 492 (M^+, 0·1%), 372, 330, 312, 302, 285, 270, 260, 257, 247, 243, 242, 229, 216, 215, 147, 120 (37%), and 43 (100%),* δ [100 MHz; $(CD_3)_2CO$] 7·3 and 7·1 (AB, *S* 9, arom.), 3·8, 3·1, and 2·9 (ABX, *S* 5, 5, and 18, lactone A), 5·4 and 4·1 (AB, *S* 7, lactone B), 5·3 and 4·2 (A_2B, *S* 6, side-chain), and 2·26, 2·18, 2·09, and 2·00 (4 A_3, acetate).

(b) *From conocarpin.* Conocarpin (161 mg) was kept for 5 min in 0·1N-sodium hydroxide (10 ml; pH of solution *ca.* 9); the solution was evaporated at 0·1 Torr to give the salt as a clear glass (211 mg), $\nu_{max.}$ 1560—1600 cm^{-1} (CO_2^-) (no other carbonyl absorption at 1700—1800 cm^{-1}). The salt (108 mg) was shaken for 16 h with pyridine (1 ml) and acetic anhydride (2 ml); the mixture was filtered and the filtrate dried to leave a residue (148 mg) showing two major components on t.l.c. in benzene–butanone (3 : 1 v/v) with R_F 0·58 (for conocarpin tetra-acetate) and 0·51 (for *allo*-conocarpin tetra-acetate) and showing distinctly different colours on heating the plate after spraying with chromic acid reagent.[16] An oily component separated from a solution in acetic acid, which then furnished *allo*-conocarpin tetra-acetate (50 mg), m.p. 188—190° (Found: C, 56·0; H, 4·8%), identical (mixed m.p. and i.r. spectra) with the material obtained in (a).

(+)-p-*Methoxyphenylsuccinic Acid from Conocarpic Acid.*—Conocarpic acid (200 mg) in methanol (5 ml) was treated with 0·2M-diazomethane in ether (40 ml) for 16 h. The product, a syrup (213 mg) showing six components on t.l.c., was directly oxidised by keeping it in 2N-sodium hydroxide (3 ml), water (50 ml), and sodium periodate (1·9 g in 20 ml of water) for 4 h at 32°. Excess of sulphur dioxide gas was passed through and the solution extracted continuously (16 h) with ether to yield a solid which from water gave (+)-*p*-methoxyphenylsuccinic acid (20 mg), m.p. 198—201° (Found: C, 58·8; H, 5·5. Calc. for $C_{11}H_{12}O_5$: C, 58·9; H, 5·4%), $[\alpha]_D$ +127° (*c* 1·01) (lit.,[1] m.p. 200·5—201·5°, $[\alpha]_D$ +127°).

Reflexin Methyl Ether (X).—Reflexin (324 mg) in methanol (3 ml) was treated with 0·2M-diazomethane in ether (9 ml) for 1 h. Acetic acid (0·5 ml) was added, the solution was evaporated, and the residue crystallised from water to give crystals (50 mg), m.p. 173—175° (Found: C, 55·1; H, 6·0. $C_{17}H_{22}O_9$ requires C, 55·1; H, 6·0%), $[\alpha]_D$ +44° (*c* 0·85), $\nu_{max.}$ 3440, 3370, and 3110 (OH) and 1765 and 1730 cm^{-1} (C=O), *m/e* 370 (M^+, weak), 338, 193, 161, 151, and 134 (100%), identical (i.r. spectrum) with the compound obtained [1] from conocarpin.

Reflexin Spiro-dibromoquinolide Ether (XVII).—Reflexin (500 mg) in water (0·5 ml) was treated with saturated bromine water (18 ml). A colourless solid crystallised after 1 min and was recrystallised from water; yield 400 mg, m.p. 143—145° (Found: C, 37·3; H, 3·3. $C_{16}H_{16}Br_2O_9$ requires C, 37·5; H, 3·1%), $\lambda_{max.}$ (50% EtOH) 205 and 261 nm (ε 6000 and 10,700), $\lambda_{max.}$ (0·1N-NaOH in 50% EtOH) 217, 240, and 321 nm (ε 18,000, 7500, and 4000); $\nu_{max.}$ 3510 and 3390 (OH), 1792, 1765, 1730, 1710, 1698sh, and 1685 (C=O), and 1602 cm^{-1} (C=C), *m/e* 510, 512, and 514 (M^+, weak); 478, 480, and 482; 402; 399 and 401; 321 and 323; 295, 278; 266, 268 (100%), and 270; 153, and 125, δ [60 MHz; $(CD_3)_2CO$] 7·8 and 7·7 (AB, *S* 2, olefinic), 3·3, 2·8, and 2·3 (ABX, *S* 9, 5, and 16, ester side-chain), 5·1 and 4·7 (AB, *S* 4, lactone ring), 4·0 and 3·8 (A_2B, *S* 5, glycol side-chain), 3·57 (A_3, OCH_3), and 5·9, 4·6, and 3·2 (3A, OH). Its *triacetate* (XVIII) was obtained as a glass either from pyridine and acetic anhydride as before, or as a glass (270 mg distilled at 260° and 0·1 Torr, single spot on t.l.c.)

* This completely different fragmentation pattern *vis-à-vis* conocarpin tetra-acetate accords with the *ortho*-fused (for the first compound) as against the spiro-fused dilactone system (for the latter).

[16] H. Ertel and L. Horner, *J. Chromatog.*, 1962, **7**, 268.

by leaving it (250 mg) in acetic anhydride (10 ml) and boron trifluoride–ether (1 ml) for 16 h (Found: C, 41·4; H, 3·4. $C_{22}H_{22}Br_2O_{12}$ requires C, 41·4; H, 3·5%); $\nu_{max.}$ 1800, 1750, and 1692 (C=O), and 1602 cm^{-1} (C=C), *m/e* 636, 638, and 640; 594, 596, and 598; 371; 335, 337, and 339; 293, 295, and 297; 268, 166, 113, 107, 103, and 59 (100%), δ(60 MHz; $CDCl_3$) 7·3 and 7·3 (AA′, olefinic), 3·3, 2·8, and 2·5 (ABX, *S* 9, 4·5 and 16), 5·1 and 4·7 (AB, *S* 4·5), 5·4 and 4·3 (A_2B), 2·24, 2·13, and 2·05 ($3A_3$, acetate), and 3·57 (A_3, OCH_3).

Conocarpin Spiro-dibromoquinolide Ether (XXI).—(*a*) Conocarpin (300 mg) in water (1 ml) and saturated bromine water (15 ml) afforded a gel which on warming gave crystals of the *hydrate* of (XXI) (270 mg), m.p. 215—218° (from aqueous methanol) (Found: C, 36·4; H, 2·8; O, 28·8. $C_{15}H_{12}Br_2O_8,H_2O$ requires C, 36·2; H, 2·8; O, 28·9%), $\lambda_{max.}$ (50% EtOH) 260, 295, and 320 nm (ε 8500, 1200, and 600), $\lambda_{max.}$ (0·1N-NaOH in 50% EtOH) 218, 240, and 322 nm (ε 18,400, 8600, and 4600), $\nu_{max.}$ 3530 and 3420 (OH), 1819, 1780, and 1680 (C=O), and 1602 cm^{-1} (C=C), *m/e* 478, 480, and 482 (M^+, weak); 399 and 401; 266, 268 (100%), and 270; 186 and 188; 158 and 160; and 125, δ[100 MHz, $(CD_3)_2CO$] 7·56 and 7·64 (AB, *S* 2·5, olefinic), 3·8, 3·0, and 2·7 (ABX, *S* 9, 3, and 20, lactone A), 5·4 and 4·8 (AB, *S* 2, lactone B), 4·0 and 3·6 (A_2B, *S* 6, side-chain), 4·7 and 3·6 (2A, OH), and 2·8 (A, H_2O). Its *diacetate* (XXII) was obtained either from acetic anhydride and pyridine solution as before, or by keeping it (300 mg) in acetic anhydride (10 ml) and concentrated sulphuric acid (0·1 ml) for 16 h; chloroform (10 ml) was then added and this solution washed with water and dried; the product distilled at 250° and 0·1 Torr as a glass (300 mg) (Found: C, 40·5; H, 2·7. $C_{19}H_{16}Br_2O_{10}$ requires C, 40·4; H, 2·8%). Its 10,11-*dimethyl acetal* (XXIII) was obtained by leaving it (300 mg) in dry acetone (100 ml) over anhydrous copper sulphate (8 g) for 16 h and chromatographing it in benzene–acetone (6 : 1 v/v). It was a foam, R_F 0·79 on t.l.c. in the same solvent, which did not crystallise (Found: C, 41·3; H, 3·3. $C_{18}H_{16}Br_2O_8$ requires C, 41·6; H, 3·1%), $\nu_{max.}$ 1810, 1790, and 1690 (C=O) and 1605 cm^{-1} (C=C). In pyridine and acetic anhydride no acetylation occurred and unchanged acetal was recovered; thus no further hydroxy-groups were present.

(*b*) A solution of conocarpin methyl ether [17] (200 mg) in water (3 ml) and saturated bromine water (15 ml) was kept for 48 h and then concentrated to 3 ml; the product crystallised (140 mg) and was recrystallised from aqueous methanol to give the same material as before (110 mg), m.p. 215—218° (Found: C, 36·3; 36·2; H, 2·5, 2·7. Calc. for $C_{15}H_{12}Br_2O_8,H_2O$: C, 36·2; H, 2·8%), with the same u.v., i.r., and mass spectral data.

3-(4-*Hydroxyphenyl*)*propan*-1-*ol* (XXVI).—*p*-Hydroxycinnamic acid (9·9 g) in dry ether (500 ml) was refluxed with lithium aluminium hydride (15 g) for 72 h; 10% sulphuric acid (400 ml) was slowly added and the solution was extracted continuously with ether to yield 3-(4-hydroxyphenyl)prop-2-en-1-ol (5·1 g), m.p. 120—121° (lit., m.p. 124°), as crystals from aqueous ethanol. This alcohol (2 g) in ethanol (15 ml) was hydrogenated over platinum oxide (50 mg) for 1 h at 1 Torr; the product was chromatographed in benzene–ethyl acetate (7 : 3 v/v) and crystallised from ether–light petroleum; yield 910 mg, m.p. 48—52° (lit., m.p. 55°) (Found: C, 71·0; H, 7·9. Calc. for $C_9H_{12}O_2$: C, 71·1; H, 7·9%), $\nu_{max.}$ 3450 and 3260 cm^{-1} (OH), $\lambda_{max.}$ 223 and 282 nm (ε 6150 and 1480).

7,9-*Dibromo*-1-*oxaspiro*[4,5]*deca*-6,9-*dien*-8-*one* (XXVII).—3-(4-Hydroxyphenyl)propan-1-ol (152 mg) in ethyl acetate (5 ml) was shaken with bromine (960 mg) and water (5 ml) for 2 h. The product was recovered from the organic phase and chromatographed in benzene (in which it had R_F 0·30) to yield a *solid* which crystallised from aqueous methanol; yield 150 mg, m.p. 119—120° (Found: C, 35·1; H, 2·6. $C_9H_8Br_2O_2$ requires C, 35·1; H, 2·6%), $\lambda_{max.}$ (MeOH) 204 and 257 nm (ε 6450 and 9200), $\nu_{max.}$ 1690 (C=O) and 1600 cm^{-1} (C=C), *m/e* 306, 308, and 310 (M^+, weak); 227 and 229 (both 100%, M − Br); 199 and 201 (M − Br − CO); and 120 (M − Br_2 − CO), δ($CDCl_3$) 7·2 (AA′, olefinic), 2·1 (4H, m, C_2H_4 chain on ring), 4·0 (2H, t, CH_2·O).

Bromination Reactions of 3-(4-*Hydroxyphenyl*)*propan*-1-*ol*.—(a) *In methanol*. 3-(4-Hydroxyphenyl)propan-1-ol (152 mg) and bromine (960 mg) were kept in methanol (15 ml) for 1 h. The oil obtained on evaporation was chromatographed in benzene–butanone (7 : 3 v/v) and the resultant oil (150 mg; t.l.c. R_F 0·52 in the same solvent) distilled at 170° and 0·3 Torr. The product (120 mg) (Found: C, 34·7; H, 3·2. $C_9H_{10}Br_2O_2$ requires C, 34·8; H, 3·2%) was 3-(3,5-*dibromo*-4-*hydroxyphenyl*)*propan*-1-*ol* (XXVIII), $\lambda_{max.}$ (MeOH) 211, 221sh, 284, 290, and 310sh nm (ε 28,800, 18,200, 5300, 5500, and 2000), $\nu_{max.}$ 3530 and 3380 cm^{-1} (OH), *m/e* 308, 310, and 312 (M^+); 290, 292, and 294; 263, 265, and 267; 185 and 187; and 132 (100%), δ($CDCl_3$) 7·3 (A_2, arom.), 3·6, 2·6, and 1·8 ($A_2M_2X_2$ in side-chain), and 4·5br (2H, OH).

(b) *In acetic acid*. 3-(4-Hydroxyphenyl)propan-1-ol (152 mg) and bromine (960 mg) were kept in acetic acid for 2 h. The syrup obtained on evaporation was chromatographed in benzene (t.l.c. R_F 0·30 in benzene) and yielded a solid, crystallised (yield 220 mg) from acetic acid, m.p. 68—71° (Found: C, 37·2; H, 3·4. $C_{11}H_{12}Br_2O_3$ requires C, 37·5; H, 3·4%), and identified as 3-(3,5-*dibromo*-4-*hydroxyphenyl*)*propyl acetate* (XXIX), $\lambda_{max.}$ (MeOH) 212, 222sh, 285, 292, and 312sh nm (ε 21,100, 13,900, 3500, 3600, and 1700), $\nu_{max.}$ 3430 (OH) and 1725 cm^{-1} (ester C=O), *m/e* 350, 352, and 354 (M^+); 290, 292 (100%), and 294 (M − 60, hence side-chain acetate), 263, 265, and 267; and 132, δ($CDCl_3$) 7·3 (A_2, arom.), 4·1, 2·6, and 1·9 ($A_2M_2X_2$), 5·8 (A, OH), 2·06 (A_3, acetate). The acetate (100 mg) was hydrolysed by refluxing in ethanol (10 ml) and 2M-hydrochloric acid (5 ml) for 2 h and distilling the recovered oil at 170° and 0·3 Torr, to give the phenol-alcohol (XXVIII) (80 mg) (Found: C, 34·7; H, 3·3), identified by i.r. spectra and t.l.c. The monoacetate (100 mg) was also acetylated in acetic anhydride (2 ml) and pyridine (1 ml) for 16 h to give 3-(4-*acetoxy*-3,5-*dibromophenyl*)*propyl acetate* (XXX), an oil, b.p. 175° at 0·4 Torr, R_F 0·20 on t.l.c. in benzene (90 mg) (Found: C, 39·5; H, 3·6. $C_{13}H_{14}Br_2O_4$ requires C, 39·6; H, 3·6%), $\lambda_{max.}$ (MeOH) 220, 273, and 282 nm (ε 26,600, 1580, and 1510), $\nu_{max.}$ 1780 and 1740 cm^{-1} (aryl and alkyl acetate), δ($CDCl_3$) 7·4 (A_2, arom.), 4·1, 2·6, and 1·9 ($A_2M_2X_2$), and 2·38 and 2·04 (A_3, acetate).

The Dibromo-hydroxychroman [(XXXI) *or* (XXXII)].—The model dibromoquinolide (XXVII) (100 mg) and boron trifluoride–ether (1 ml) were kept in benzene (10 ml) for 16 h. Water (10 ml) was added and the product was recovered from the benzene layer. It had R_F 0·62 (t.l.c. in benzene) and was chromatographed in benzene to yield the chroman (XXXI) or (XXXII) (70 mg), m.p. 79° (from aqueous ethanol) (Found: C, 35·0; H, 2·6. Calc. for

[17] Ref. 1, p. 2132.

$C_9H_8Br_2O_2$: C, 35·1; H, 2·6%), $\nu_{max.}$ 3465 cm^{-1} (OH), *m/e* 306, 308 (100%), and 310 (M^+); 278, 280, and 282 ($M - C_2H_4$, by retro-Diels-Alder loss [18] of ethylene from the heterocycle); 250, 252, and 254 ($M - C_2H_4 - CO$); [19] and 148, δ($CDCl_3$) 7·0 (A, arom.), 4·1, 2·7, and 2·0 ($A_2M_2X_2$), and 6·5 (A, OH).

The Bis(dibromo-hydroxychroman) [(XXXIII) *or* (XXXIV)].—The model dibromoquinolide (XXVII) (200 mg) was slowly dissolved in concentrated sulphuric acid (4 ml) at 0°, kept at 0° for 30 min, and poured into ice-water (10 ml); the product was extracted with ether as a red solid which showed only one spot at R_F 0·74 on t.l.c. in benzene–ethyl acetate (97 : 3 v/v). It was chromatographed in this solvent and afforded a crystalline solid (80 mg), m.p. 198—200° (from ethanol) (Found: C, 35·4, 35·4; H, 2·4, 2·3. Calc. for $C_{18}H_{14}Br_4O_4$: C, 35·2; H, 2·3%), regarded as the bichroman (XXXIII) or (XXXIV), $\lambda_{max.}$ (MeOH) 210 and 310 nm (ε 22,800 and 4500), $\nu_{max.}$ 3465 and 3430 cm^{-1} (OH), *m/e* 610, 612, 614 (100%), 616, and 618 (M^+); 531, 533, 535, and 537 (M — Br); 503, 505, 507, and 509 (M — Br — C_2H_4); [18] 475, 477, 479, and 481 (M — Br — $2C_2H_4$); [18] and 149,* δ($CDCl_3$) 4·0, 2·7, and 2·0 ($2A_2M_2X_2$), and 6·6 (2A, OH) as the only proton signals.

* The ions of *m/e* 503, 505, 507, and 509 were shown by accurate *m/e* determination to correspond to M — Br — C_2H_4 and not to M — Br — CO [Found: *m/e* 504·8081. $C_{16}H_{10}{}^{79}Br_2{}^{81}BrO_4$ requires *m/e* 504·8108; $C_{17}H_{14}{}^{79}Br_2{}^{81}BrO_3$ requires *m/e* 504·8472]. The ions at *m/e* 475, 477, 479, and 481 correspond to M — Br — $2C_2H_4$ and not to M — Br — C_2H_4 — CO [Found: *m/e* 476·7788. $C_{14}H_6{}^{81}Br_2{}^{81}BrO_4$ requires 476·7795; $C_{15}H_{10}{}^{79}Br_2{}^{81}BrO_3$ requires 476·8159], and thus demonstrate the presence of the bichroman system.

We thank Professor H. B. Rycroft, National Botanic Gardens of South Africa, for a supply of plant material, the Council for Scientific and Industrial Research for an assistantship (to G. W. P.), Mr. H. K. L. Hundt for his assistance, and Drs. S. H. Eggers and K. G. R. Pachler of the C.S.I.R. for obtaining mass and n.m.r. spectra.

[2/462 *Received*, 28*th February*, 1972]

[18] C. Djerassi, H. Budzikiewicz, and D. H. Williams, 'Interpretation of Mass Spectra of Organic Compounds,' Holden-Day San Francisco, 1964, pp. 102 and 236.

[19] Ref. 18, pp. 171 and 178.