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THE STRUCTURE OF SCLERIN, A METABOLITE OF *SCLEROTINIA LIBERTIANA*[1]

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Abstract—The structure of sclerin, a new type of plant growth regulator, is shown to be II.

SCLERIN is a metabolite of *Sclerotinia libertiana* which was isolated originally as lipase formation stimulating factor in the mycelium.[2] Later study on the physiological activities of this compound has disclosed that it promotes the enzyme formation and growth of various plant seedlings, thus functioning as a plant growth hormone. The feature and scope of this effect has been characterized.[2]

Sclerin, $C_{13}H_{14}O_4$, m.p. 123°, $[\alpha]_D$ ~0°* gave a monoacetate, m.p. 156° upon acetylation with acetyl chloride and pyridine. The presence of molecular peaks at *m/e* 234 and 276 respectively in the mass spectra of both compounds provided a support for the assigned molecular formula of sclerin. The phenolic nature of sclerin was further revealed by positive ferric chloride test and the presence of IR peak at 3260 cm^{-1}, which indicated the involvement of the OH group in a chelated structure. In accordance the OH proton has a signal in the NMR spectrum at such a low field as −0·80 τ. The presence of the remaining O atoms as an anhydride grouping was assumed from the IR peaks at 1790 and 1735 cm^{-1} in the acetate III and at 1785 and 1738 cm^{-1} in the methyl ether IV (cf. later for derivation). One of the CO groups in the anhydride structure must participate in the formation of the chelated ring with the phenolic OH group mentioned above. Sclerin exhibits absorption bands in UV region at λ_{max} 215·5, 263 and 333 mμ (ε 23,100, 7900 and 3500), indicative of a conjugated benzene chromophore. Necessarily, one end of the anhydride group has to be attached directly to the aromatic ring and this, in conjunction with the presence of chelation, reasonably explains a relatively wide separation of anhydride twin band in sclerin (1800 and 1690 cm^{-1}) and the partial structure I was derived for sclerin. In the NMR spectrum sclerin showed signals, in addition to the peak due to the OH proton, at 7·72, 7·78 and 7·83 τ (each 3H, s) due to three Me groups on an aromatic ring and signals at 8·47 (3H, d, J = 7 c/s) and 5·86 τ (1H, q, J = 7 c/s) due to a

MeCH-group. The structural units thus revealed are accounted for satisfactorily in the partial formula I and this led to the formulation of sclerin as II. The calculated position of major electron transfer band according to the proposed empirical rule for

* In the preliminary paper, we reported $[\alpha]_D$ of +7·85° ($CHCl_3$) for sclerin. It was later found that sclerin was obtained mostly as a racemate, and, otherwise specified, the designation as sclerin in this work refers to the racemic material. Optically active sclerin ($[\alpha]_D$ +18·5°) was also isolated in minor amounts from the mother liquor of racemic sclerin fraction.[3]

aromatic carboxylic acids[4]* is 255 mμ, which is in good agreement with the observed value of 263 mμ, if allowance is made for the structural differences involved.

The various transformations of sclerin could be reasonably interpreted in terms of structure II and confirmed this structure. Sclerin was recovered unchanged after hydrolysis with alkali and subsequent acidification. The dimethyl ester V, m.p. 116°, ν_{max} 3200, 1720, 1670 cm^{-1}, obtainable through treatment of sclerin with methanolic hydrogen chloride, also regenerated II upon alkaline hydrolysis. This unusual stability of the anhydride ring appears, however, to be without precedent. This is probably due to the relatively rigid anhydride ring structure being favoured by the fixed conformation of the nuclear carboxyl group due to hydrogen bonding on one hand and by the restriction imposed on the rotation of the 1-(1-carboxy)ethyl side chain due to the interfering Me group in *peri*-position on the other hand. Blocking of hydrogen bonding by methylation of the phenolic OH group results in destruction of the ring stability and thus, the diacid XIII no longer had a tendency to close the anhydride ring spontaneously in an acidic medium (see below). Methylation of sclerin with dimethylsulfate and potassium carbonate in acetone furnished, after chromatographic separation, an oily methyl ether dimethyl ester (VI), ν_{max} 1725 cm^{-1} and a small amount of crystalline substance (VIII), m.p. 169°, ν_{max} 1755, 1725 cm^{-1}. VI had three Me singlets at 6·22, 6·37 and 6·43 τ in the NMR spectrum and, when treated with alkali, afforded a methyl ether methyl ester acid (VII), m.p. 97°, ν_{max} 1725 cm^{-1}, $\nu_{max}^{CHCl_3-Et_3N}$ 1720, 1590 cm^{-1}, which showed NMR signals at 0·17 (1H, s, —CO_2H), 6·10 and 6·26 τ (each 3H s, —OMe and —CO_2Me). In VII the nuclear carbomethoxy group was left unhydrolyzed. The hindered nature of this group was also evident in the UV spectrum of VII, where the major electron transfer band was undiscernible by the steric inhibition of resonance (cf. Experimental). VIII exhibited in the NMR spectrum pairs of signals at 5·00 and 6·51 τ (1H, q, J = 7 c/s) and, at 8·41 and 8·45 τ (3H, d, J = 7 c/s) due to $MeCH(-O-)$— groups, suggesting that VIII is an unsymmetrical dimer. A phenol ester linkage in VIII was indicated by the presence of a carbonyl absorption at 1755 cm^{-1}. Therefore, it is concluded that VIII results from self-acylation of II, which would lead to two possible products, VIII and VIIIa. The correct structure (VIII) was ascertained by the hydrolysis experiment, which afforded sclerin (II) and the methyl ester acid VII in comparable amounts as expected from the difference in the behaviour of V and VI on alkaline hydrolysis. The remaining ester group of VI could be hydrolysed by treatment with hydrochloric acid in boiling acetic acid to give the afore-mentioned dicarboxylic acid XIII, m.p. 78°, together with the nor acid methyl ether XXX, which is produced through decarboxylation of the nuclear carboxyl group. XIII was converted to sclerin methyl ether (IV) by treatment with boiling acetic anhydride.

In agreement with the formulation as an anhydride, treatment of sclerin with phenyl hydrazine or semicarbazide yielded a phenylhydrazide (IX), m.p. 195°, ν_{max} 3400, 3350, 1720, 1640 cm^{-1}; λ_{max} 274, 342 mμ (ε 12,700, 5600) and a semi-

* The electron transfer band of salicylic acid at 236 mμ was chosen as the basis of calculation. IV, XIV, XV, XIX and sclerolide (XXXIII) have these bands at 261, 260·5, 253, 248 and 250 mμ, respectively. The presence of absorption maxima at lower wave lengths in the latter two can be ascribed to the ring strain effect.[4] Band positions of the major electron transfer absorption of substituted aromatic acids and esters have been compared, and it has been found that the latter absorbs at somewhat higher wave length (2–6 mμ).[5]

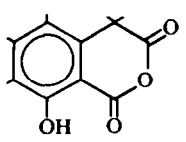
carbazide (X), m.p. 227°; ν_{max} 3500, 3300, 3200, 1730, 1680, 1650 cm^{-1}; λ_{max} 273, 340 mμ (ε 11,100, 5200) respectively, instead of a hydrazone or semicarbazone. In the same way, treatment of sclerin with ammonia followed by pyrolysis furnished a lactim (XII), m.p. 229°, ν_{max} 3180, 3060, 1700, 1660, 1615 cm^{-1}; λ_{max} 210, 270, 339 mμ (ε 25,300, 8900, 3600), λ_{infl} 224 mμ (ε 13,100), which also was obtained by acid treatment of an amide (XI), m.p. 113°; ν_{max} 3400, 3300, 3220, 1735, 1720, 1690 cm^{-1}; λ_{max} 212·5, 283 mμ (ε 19,900, 2100) obtainable from VII. The MeO group was concurrently eliminated in the latter reaction. The relative ease of ether cleavage is often observed in *ortho*-alkoxybenzaldehydes or acetophenones, which is considered as the phenylogue of esters.[6]

Sclerin was reduced to a lactone (XIV), m.p. 96°, when treated with excess sodium borohydride at room temperature. It exhibited IR bands at 3130 and 1665 cm^{-1}, and UV maxima at 217, 260 and 330 mμ (ε 27,700, 10,100 and 3900), which suggest reduction of the aliphatic carboxyl of the anhydride ring and the preservation of the the chelated system. This was substantiated by the NMR spectrum, which showed signals at −1·49 (1H, s, chelated OH), 5·57 (2H, m, $-\underset{|}{C}HCH_2OCO-$) and 6·86 τ (1H, m, $-\underset{|}{C}HCH_2OCO-$). As expected from the greater degree of the hydrogen bonding, the phenolic OH group of the lactone XIV was more difficult to methylate than sclerin, and XIV gave a methyl ether (XV), m.p. 134°; ν_{max} 1710 cm^{-1}, λ_{max} 213, 253, 305 mμ (ε 36,000, 9100, 2400) only under forcing conditions.[7] Sodium borohydride treatment of sclerin at an elevated temperature led to the formation of a cyclic ether (XVI), m.p. 134°; ν_{max} 3400, 1610, 1585 cm^{-1}, λ_{max} 212, 280 mμ (ε 13,100, 700). In addition to NMR signals in XIV, the appearance of an AB quartet at 4·99 and 5·38 τ (J = 16 c/s) in XVI clearly indicated the conversion of the lactone carbonyl in XIV to a methylene. Reduction of anhydrides[8,9] and lactones[8–10] by the action of sodium borohydride or diborane has been observed previously.

The structure of the anhydride moiety of sclerin was clarified by decarboxylation reactions of the half ester VII. Curtius degradation of VII produced a 5-membered lactam (XVII), m.p. 210°, with small amounts of a basic product (XVIII), m.p. 102°. XVII exhibited IR peaks at 3190, 3060 and 1690 cm^{-1} and a quartet at 5·35 τ (J = 7 c/s) attributable to the methine proton, verifying the assigned formulation. The structure XVIII is suggested for the basic byproduct of the degradation on the basis of spectroscopic evidence.* Thus it had absorption bands at 3400, 3310 (—NH_2) and 1735 cm^{-1} (conjugated γ-lactone) in the IR spectrum and at λ_{max} 249·5, 297 and λ_{infl} 290·5 mμ (ε 11,000, 3300 and 3100) in the UV region. In the NMR spectrum, XVIII was devoid of signals due to a $MeCH(-)$ group and instead had a Me singlet at 8·20 τ. In addition, the amino protons were observed as a broad signal superimposed with the one due to three aromatic Me groups at 7·55 and 7·72 τ.

Reaction of VII with lead tetraacetate in acetic acid[12] followed by alkaline hydrolysis led to the formation of a 5-membered lactone (XIX), m.p. 90°, ν_{max} 1745 cm^{-1}. A quartet (J = 7 c/s) due to a $MeCH(-)$ group was found at 4·54 τ in its NMR spectrum, and consistent with the formulation as XIX.

* XVIII is clearly preferable to the alternate hydroxy-lactone formulation XVIIIa from the value of the carbonyl stretching frequency in the IR spectrum.[11]



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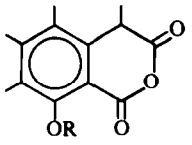
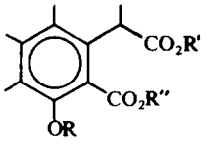
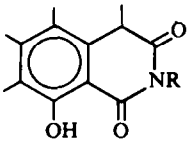


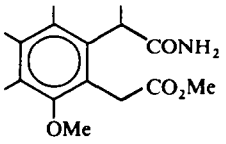
II: R = H
III: R = Ac
IV: R = Me



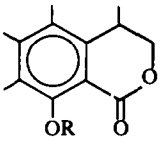
V: R = H, R′ = R″ = Me
VI: R = R′ = R″ = Me
VII: R = R″ = Me, R′ = H
XIII: R = Me, R′ = R″ = H



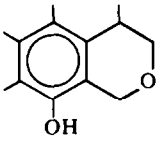
IX: R = NHC_6H_5
X: R = $NHCONH_2$
XII: R = H



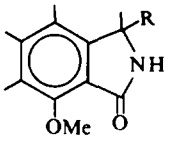
XI



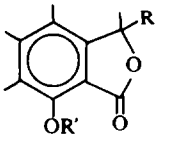
XIV: R = H
XV: R = Me



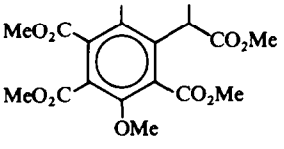
XVI



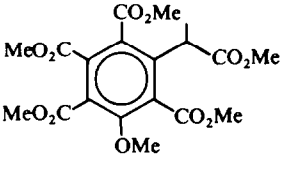
XVII: R = H
XVIIIa: R = OH



XVIII: R = NH_2, R′ = Me
XIX: R = H, R′ = Me
XXXIII: R = R′ = OH

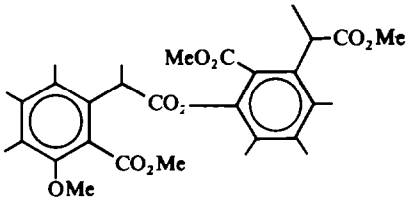


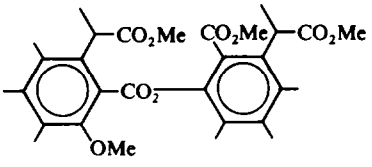
XX



XXI

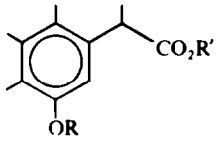
The evidence pertaining to the aromatic substitution pattern will now be discussed. On exposure to alkaline potassium permanganate at 80°, the half ester VII furnished, after methylation with diazomethane and subsequent chromatography of the resulting esters, a tetracarboxylic acid methyl ester (XX), m.p. 88° and a pentacarboxylic acid methyl ester (XXI), m.p. 92·5°, leaving the 1-(1-carboxy)ethyl side chain intact. One of the ring Me groups remained unattacked in the former as revealed by a signal at 7·39 τ (s, 3H) in its NMR spectrum and this would be at the most hindered position, namely at carbon 5. The presence of at least two adjacent Me groups on the aromatic ring was indicated by ozonolysis of sclerin, which afforded diacetyl, characterized as its mono-2,4-dinitrophenylhydrazone. Treatment of sclerin with 40% KOHaq in a



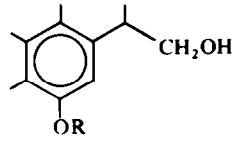


VIIIa

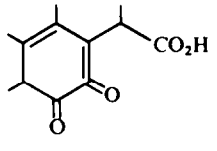
sealed tube at 180° caused the decarboxylation of the nuclear carboxyl group to give a nor acid (XXII), m.p. 126°; ν_{max} 3700, 3300–2450, 1705, 1600, 860 cm^{-1}; λ_{max} 280·5, 290, λ_{infl} 222, 284 mμ (ε 24,200, 2400, 9400, 2300). On methylation with dimethyl sulfate, it afforded a methyl ether methyl ester (XXIII), m.p. 42·5°, the NMR spectrum of which was in agreement with the depicted structure for the degraded acid; 3·52 (s, ArH), 6·12 (q, J = 7 c/s, —C<u>H</u>Me), 6·26, 6·24 (each s, —OMe and —CO_2Me), 7·83 (s, 2 ArMe), 7·91 (s, ArMe), 8·61 τ (d, J = 7 c/s, <u>Me</u>CH—). LAH reduction of XXIII gave a primary alcohol (XXIV), m.p. 52°. Short treatment of the nor acid XXII with diazomethane resulted in the formation of a hydroxy methyl ester (XXV), m.p. 115°, ν_{max} 3450, 1710, 1590, 850 cm^{-1}, while acetylation of XXII gave an acetate (XXVI),



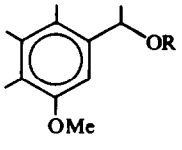
XXII: R = R′ = H
XXIII: R = R′ = Me
XXV: R = H, R′ = Me
XXVI: R = Ac, R′ = H
XXX: R = Me, R′ = H



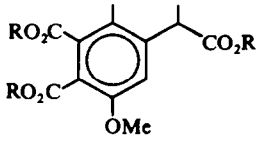
XXIV



XXVII



XXXI: R = Ac
XXXII: R = H



XXVIII: R = H
XXIX: R = Me

m.p. 113°. That one of the positions *ortho* to the phenolic hydroxyl in XXII is free, was confirmed by the preparation of an *o*-quinone (XXVII) through oxidation of the nor acid XXII with potassium nitrosodisulphonate. The product had IR peaks at 3200–2400, 1700, 1660, 1640 and 1570 cm^{-1}, and UV absorption bands at λ_{max} 440 and λ_{infl} 263 mμ (ε 1600 and 2900). These properties bear a close resemblance to those of tetramethyl-*o*-benzoquinone,[13] ν_{max} 1660, 1623, 1350 cm^{-1}, λ_{max} 447 mμ (ε 1140). Analogous oxidation of XXIII with permanganate as in VII furnished a tricarboxylic acid (XXVIII), characterized as its trimethyl ester (XXIX), m.p. 65·5°. Again the 1-carboxyethyl side chain was resistant to the action of the oxidizing agent. Final proof of the substitution pattern on the aromatic ring of sclerin was provided by the degradation of the nor acid XXII. The methyl ether acid (XXX), m.p. 132°, obtained by alkaline hydrolysis of the methyl ether methyl ester XXIII was subjected to lead tetraacetate treatment in acetic acid followed by hydrolysis to produce the alcohol XXXII, m.p. 62°; $\nu_{max}^{CCl_4}$ 3840, 3460, 1600 cm^{-1}, together with an unidentified alcohol, m.p. 121–124°. XXXII was synthesized in a routine way starting from 2,3,4-trimethylacetophenone.[14] Both the specimens obtained by degradation and synthesis were indistinguishable in their IR spectra and on TLC plates.

Presumably sclerin is biogenetically formed by an acetate-malonate pathway.[15] The conventional arrangement of acetate units with the attachment of several C_1-units does not offer a satisfactory explanation of the construction of the metabolite. Biogenetic studies using tracers are now in progress to clarify this point.

EXPERIMENTAL

M.ps were uncorrected. The IR spectra were run, unless otherwise stated, on Nujol mull on a Nippon Bunko IR–S spectrometer and the UV spectra were determined in EtOH soln on a Hitachi EPS–2 recording spectrometer. The NMR spectra were taken in $CDCl_3$ soln on a JNMC–60 or Varian A–60 spectrometer. Chemical shifts are reported in τ-values, using TMS as internal references. Mallinckrodt silicic acid was used for column chromatography and silica gel G acc. to Stahl, Merck for TLC. Microanalyses were carried out at Microanalytical Laboratory, Faculty of Science, Osaka City University.

Sclerin (II)

Crude material provided from Prof. Y. Satomura (obtained by extracting the mycelium and the culture medium of *Sclerotinia libertiana* with AcOEt)[2] was purified through silica gel chromatography and recrystallization from a mixture of $CHCl_3$ and ether. M.p. 123°. $[\alpha]_D$ ~0° ($CHCl_3$). $\nu_{max}^{CHCl_3}$ 3260, 1800, 1690, 1610, 1580, 1390, 1345, 1325 cm^{-1}; ν_{max} 3240, 1800, 1695 cm^{-1}; λ_{max} 215·5 (ε 23,100), 263 (7900), 333 mμ (3500). $\lambda_{max}^{0·01NKOH-EtOH}$ 313 (ε 4200), $\lambda_{infl.}$ 246 mμ (6100); NMR: −0·80 (1H, s.), 5·86 (1H, q., J = 7 c/s), 7·72, 7·78, 7·83 (each 3H s), 8·47 (3H, d, J = 7 c/s). M.S. (rel. int.)*: 234 (46·9), 206 (100), 44 (61·3), 28 (82·9), 18 (53·6). It gave a greenish blue colour in the $FeCl_3$ test (MeOH). Sclerin dissolves gradually in $NaHCO_3$ aq and ammonia. Sclerin was recovered unchanged when an alkaline soln was acidified.

Sclerin acetate (III)

A cooled soln of sclerin (100 mg) in pyridine (1·2 ml) was mixed with acetyl chloride (0·4 ml) and kept at 0° for 1 hr and at room temp for 4 hr. The neutral ether extract (134 mg) was recrystallized from ether to give prisms, m.p. 156°; ν_{max} 1790, 1760, 1735, 1585, 1195 cm^{-1}; λ_{max} 216 (ε 25,500), 260 (8500), 313 mμ (2600). NMR: 5·85 (1H, q, J = 7 c/s), 7·58 (3H, s, $MeCO_2$—), 7·66, 7·72, 7·81 (each 3H, s, 3ArMe), 8·44 (3H, d, J = 7 c/s). (Found: C, 65·59; H, 6·10. $C_{15}H_{16}O_5$ requires: C, 65·21; H, 5·84%.) When refluxed with alcoholic NaOH, the acetate gave back sclerin in nearly quantitative yield.

* Measured on a Hitachi Model RMU–6 mass spectrometer; ionizing voltage, 80 eV; vaporizing temp, 180°

*Sclerin p-brombenzoate**

A soln of sclerin (100 mg) in dry pyridine (2·0 ml) was refluxed with *p*-brombezoyl chloride (246 mg) for 4 hr. An oily product (92 mg) obtained by the usual work up was passed through a column of silica gel (2·0 g) and the $CHCl_3$ eluate was recrystallized from AcOEt to give prisms, m.p. 198–199°; ν_{max} 1785, 1745 cm^{-1}. (Found: C, 57·58; H, 4·27. $C_{20}H_{17}O_5Br$ requires: C, 57·57; H, 4·11%.)

Hydroxy dimethyl ester (V) (*methyl 1-(5-hydroxy-6-methoxycarbonyl-2,3,4-trimethylphenyl)propionate*)

A soln of sclerin (154 mg) in abs MeOH (15 ml) was saturated with dry HCl under ice-cooling and then allowed to stand overnight at room temp. The concentrated reaction mixture was taken up in $CHCl_3$ and washed with $NaHCO_3$ aq. The product was recrystallized from $CHCl_3$–pet. ether to give needles, m.p. 113–116°; pale green in $FeCl_3$ test; ν_{max} 3200, 1720, 1670, 1595 cm^{-1}. (Found: C, 64·60; H, 7·33. $C_{15}H_{20}O_5$ requires: C, 64·27; H, 7·19%.) When V was treated with aq EtOH–NaOH, it reverted to sclerin.

Methyl ether dimethyl ester (VI) (*methyl 1-(6-carbomethoxy-5-methoxy-2,3,4-trimethylphenyl)propionate*)

Sclerin (1·00 g) in anhyd acetone (100 ml) was heated with Me_2SO_4 (4·0 ml) and anhyd K_2CO_3 under reflux for 7 hr. After addition of Me_2SO_4 (2·0 ml), the mixture was refluxed for a further 7 hr. Filteration and concentration of the filtrate was followed by addition of ammonia to decompose excess reagent. The reaction product (1·391 g) extracted with ether was chromatographed on a column of silica gel (25 g). The first eluate with $CHCl_3$ consisted mainly of VI (728 mg) and further purified by rechromatography for the purpose of spectroscopic measurement; ν_{max} 1725 cm^{-1}. NMR: 6·22 (3H, s, —OMe), 6·37 (3H, s, $ArCO_2Me$), 6·43 (3H, s, RCO_2Me), 6·45 (1H, q, J = 7·5 c/s), 7·86 (6H, s), 7·97 (3H, s), 8·62 (3H, d, J = 7·5 c/s). The next eluate with $CHCl_3$ (326 mg) gave a crystalline dimer (VIII) on trituration with ether, which was recrystallized from ether to afford colourless prisms, m.p. 168–169°; ν_{max} 1755, 1725 cm^{-1}; λ_{max} 212·5 (ε 45,200), 283 mμ (2240); NMR: 5·00, 6·51 (each 1H q, J = 7 c/s), 6·07 (3H, s, MeO—), 6·27 (6H, s, $ArCO_2Me$), 6·37 (3H, s, RCO_2Me), 7·75, 7·89, 8·01 (each 3H s), 7·79 (9H, s), 8·41, 8·45 (each 3H, d, J = 7 c/s). (Found: C, 66·37; H, 7·34. $C_{30}H_{38}O_9$ requires: C, 66·40; H, 7·06%.)

Hydrolysis of VIII

Compound VIII (104 mg) was treated with 2N NaOH (1 ml) and MeOH (2 ml) and heated under reflux for 4 hr. The isolated product (89 mg) was recrystallized from ether–pet. ether, to give sclerin (33 mg), m.p. 121–122°. The mother liquor was chromatographed on a silica gel column (3 g). Elution with $CHCl_3$ gave, after recrystallization, VII, (31 mg), m.p. 95–97° with some of the preceding fraction of sclerin.

Methyl ether methyl ester acid (VII) (*1-(6-carbomethoxy-5-methoxy-2,3,4-trimethylphenyl)propionic acid*

Compound VI, (679 mg) was hydrolyzed by heating under reflux with 2N NaOH (20 ml) and EtOH (30 ml) for 3 hr. The acidic product (589 mg), recrystallized from benzene–pet. ether, gave VII as prisms, m.p. 98–99°; ν_{max} 1725 cm^{-1}, $\nu_{max}^{CHCl_3-Et_3N}$ 1720, 1590 cm^{-1}; λ_{max} 211 (ε 19,400), 280 mμ (1100); NMR: 0·17 (1H, s, —CO_2H), 6·10 (3H, s, —OMe), 6·26 (3H, s, —CO_2Me), 7·80 (6H, s), 7·86 (3H, s), 8·56 (3H, d, J = 7 c/s). (Found: C, 64·44; H, 7·36. $C_{15}H_{20}O_5$ requires: C, 64·27; H, 7·19%.)

Conversion of VII *to sclerin methyl ether* (IV)

A soln of VII (100 mg) in AcOH (2 ml) was treated with 3N HCl (2 ml) by heating under reflux for 6 hr. Work up in the usual way gave an oily product (75 mg), which was chromatographed on a column of silica gel (2·0 g). Elution with $CHCl_3$ gave crystalline fractions (40 mg), which were recrystallized from benzene–pet. ether to afford prisms, m.p. 128–129°, identified as *1-(5-methoxy-2,3,4-trimethylphenyl) propionic acid* (XXX), (IR). Fractions eluted with $CHCl_3$—MeOH (9:1) were recrystallized from benzene–pet. ether. The crystalline product, m.p. 77·5–78·5°; ν_{max} 3600–2320, 1700 cm^{-1} represented the dicarboxylic acid (XIII) and it (19 mg) was treated with Ac_2O (1 ml) by heating under reflux for 1 hr. The residue left after evaporation of the reagent was recrystallized twice from benzene–pet. ether to give IV, m.p. 104–105°; ν_{max} 1785, 1738 cm^{-1}; λ_{max} 218 (ε 21,000), 261 (9000), 311 mμ (2500). (Found: C, 67·91; H, 6·75. $C_{14}H_{16}O_4$ requires: C, 67·73; H, 6·50%.)

Reaction of sclerin with phenylhydrazine

A mixture of sclerin (117 mg), $PhNHNH_2 \cdot HCl$ (73 mg), NaOAc (61 mg) and EtOH (10 ml) was refluxed

* X-ray crystallographic analysis has been made on this compound by A. Shimada and M. Fukuyo.

for 1 hr. A crystalline residue left after removal of the solvent gave IX, on recrystallization from EtOH, as pale yellow needles (25 mg), m.p. 194–195°. It gave a dark green colour in $FeCl_3$ test; ν_{max} 3400, 3350, 1720, 1640 cm^{-1}; λ_{max} 274 (ε 12,700), 342 mμ (5600); NMR: −1·58 (1H, s, chelated —OH), 4·86 (1H, s, —NH), 5·71 (1H, q, J = 7·5 c/s), 7·69, 7·75, 7·78 (each 3H, s), 8·41 (3H, d, J = 7 c/s). (Found: C, 70·33; H, 6·28; N, 8·86. $C_{19}H_{20}O_3N_2$ requires: C, 70·35; H, 6·22; N, 8·64%.)

Reaction of sclerin with semicarbazide

Sclerin (117 mg) in aq EtOH was refluxed with $H_2NCONHNH_2 \cdot HCl$ (56 mg) and AcONa (61 mg) for 30 min. The crystalline residue obtained after evaporation of the solvent was recrystallized from EtOH to yield X as needles (41 mg), m.p. 226–227°; ν_{max} 3500, 3300, 3200, 1730, 1680, 1650, 1600 cm^{-1}; λ_{max} 273 (ε 1100), 340 mμ (5200). (Found: C, 57·78; H, 6·02; N, 14·43. $C_{14}H_{17}O_4N_3$ requires: C, 57·72; H, 5·88; N, 14·43%.)

Sodium borohydride reduction of sclerin

(a) A soln of sclerin (60 mg) in EtOH (5 ml) was added to a soln of $NaBH_4$ (60 mg) in EtOH (3 ml) and the mixture kept overnight at room temp. Dilution of the reaction mixture with water and extraction with ether yielded an oil (57 mg), which was chromatographed on silica gel column (1·5 g). The material eluted with $CHCl_3$ was crystallized from pet. ether–ether gave 8-*hydroxy*-4,5,6,7-*tetramethylisocoumarin* (XIV) as needles, m.p. 95–96°; ν_{max} 3130, 1665, 1610, 1570 cm^{-1}; λ_{max} 217 (ε 27,700), 260 (10,100), 330 mμ (3900). NMR: −1·49 (1H, s, chelated —OH), 5·57 (2H, m), 6·86 (1H, m), 7·76, 7·81, 7·85 (each 3H, s), 8·71 (d, J = 7·5 c/s). It gave a blue colour in $FeCl_3$ test. (Found: C, 70·97; H, 7·57. $C_{13}H_{16}O_3$ requires: C, 70·89; H, 7·32%.)

The lactone XIV, (134 mg, 0·61 mmole) was converted to the phenolate by stirring with NaH (50% oil dispersion, 35 mg, washed with benzene before use) in dry benzene for 1 hr. After addition of Me_2SO_4 (0·2 ml), the mixture was heated under reflux for 2 hr. Usual work up gave a neutral product (112 mg), which, on recrystallization from ether, afforded 8-*methoxy*-4,5,6,7-*tetramethylisocoumarin* (XV) as prisms, m.p. 132–134°; ν_{max} 1710 cm^{-1}, λ_{max} 213 (ε 36,000), 253 (9100), 305 mμ (2400); NMR: 5·47 (2H, m, —$\underset{|}{C}HCH_2O$—), 5·99 (3H, s, —OMe), 6·70 (1H, m, —$\underset{|}{C}HCH_2O$—), 7·64, 7·66 (9H in total, 3ArMe), 8·61 (3H, d, J = 7·5 c/s, $Me\underset{|}{C}H$—). (Found: C, 72·09; H, 7·86. $C_{14}H_{18}O_3$ requires: C, 71·77; H, 7·74%.)

(b) A soln of sclerin (600 mg) in EtOH (50 ml) was added to a soln of $NaBH_4$ (1·2 g) in EtOH (40 ml), and stirred at 40°. After being kept at this temp for 2 hr and overnight at room temp, the reaction mixture was added with dil HCl and warmed on a water bath to decompose the excess reagent and the boron compound formed. The product was extracted with ether and the ethereal extract was washed with $NaHCO_3$ aq. From the $NaHCO_3$ washings, sclerin (141 mg) was recovered upon acidification. The neutral extract (405 mg) was chromatographed on a silica gel column (9·0 g) and elution with $CHCl_3$ gave rise to XIV, (202 mg) and 8-*hydroxy*-4,5,6,7-*tetramethylisochroman* (XVI; 218 mg), after recrystallization from ether–pet. ether, needles, m.p. 133–134°; ν_{max} 3100, 1610, 1585 cm^{-1}; λ_{max} 212 (ε 13,100), 280 mμ (700); NMR 5·26 (1H, s, —OH), 4·99, 5·38 (AB quartet, J = 16 c/s), 6·14 (2H, m), 7·10 (1H, m), 7·84 (9H, s), 8·71 (d, J = 7 c/s). (Found: C, 75·66; H, 8·85. $C_{13}H_{18}O_2$ requires: C, 75·69; H, 8·80%.)

1-(6-*Carbomethoxy*-5-*methoxy*-2,3,4-*trimethylphenyl*)*propionamide* (XI)

Compound VIII (241 mg) in dry benzene (2·4 ml) was refluxed with $SOCl_2$ (0·19 ml) for 2 hr. The acid chloride thus obtained was dissolved in benzene (2·4 ml) and added dropwise to cooled and stirred conc NH_3 (2·4 ml). After stirring for 1 hr, the reaction mixture was diluted with water and extracted successively with ether and $CHCl_3$. The combined extracts were washed with sat NaCl aq and the solvent was evaporated. Chromatography of the residual oil (198 mg) on a silica gel column (4·0 g) furnished, by the elution with $CHCl_3$, crystalline fractions (148 mg), which were recrystallized from pet. ether–benzene to give XI, m.p. 112–113°; ν_{max} 3400, 3440, 3300, 3220, 1735, 1720, 1690 cm^{-1}; λ_{max} 212·5 (ε 19,900), 283 mμ (2100). (Found: C, 64·30; H, 7·46; N, 5·01. $C_{15}H_{21}O_4N$ requires: C, 64·49; H, 7·58; N, 5·01%.)

8-*Hydroxy*-4,5,6,7-*tetramethyl*-1,3-*dioxotetrahydroisoquinoline* (XII)

(a) Sclerin (117 mg) in EtOH (1·5 ml) was mixed with conc NH_3 (3·0 ml) and the mixture was evaporated to dryness under reduced press. The ammonium salt thus formed was pyrolysed with $(NH_4)_2CO_3$ (1 g) at 160° for 15 min under a N_2 atm. After addition of water, the product was extracted with ether and $CHCl_3$

to afford, after recrystallization from MeOH, XII as prisms (53 mg), m.p. 228–229°; ν_{max} 3180, 3060, 1700-1660, 1615 cm^{-1}; λ_{max} 210 (ε 25,300), 270 (8900), 339 (3600), λ_{infl} 224 mμ (13,100). (Found: C, 67·02; H, 6·66; N, 6·20. $C_{13}H_{15}O_3N$ requires: C, 66·93; H, 6·48; N, 6·01%.)

(b) The crude oily XI (20 mg) in benzene (0·5 ml) was treated with conc HCl (1 ml) by heating under reflux for 1 hr. Recrystallization of the neutral product from MeOH afforded crystals, m.p. 223°, identical with XII obtained above (IR).

Curtius degradation of VII

The acid chloride prepared from VII, (588 mg, 2·1 mmoles), by treatment with $SOCl_2$ (0·50 ml) as described above, was dissolved in dry acetone (5·9 ml) and added to a soln of NaN_3 (336 mg, 5·17 mmoles) in water under stirring and cooling in an ice bath. After stirring for 1 hr, the mixture was diluted with water and the resulting azide was extracted 3 times with benzene. The combined benzene extract was washed with water, dried with anhyd Na_2SO_4 for a short time and then concentrated to about 5 ml. The azide soln in benzene was heated gradually until the evolution of N_2 was observed at about 70°. The soln was finally heated under reflux for 1·5 hr. Conc HCl (5 ml) was added and the mixture was heated under reflux for another 1 hr. The reaction mixture was diluted with water and extracted with ether. The crystals deposited on the border of organic and aqueous layers were collected (217 mg) and recrystallized from MeOH to give *7-methoxy-3,4,5,6-tetramethyl-1-oxoisoindol* (XVII), prisms, m.p. 208–210°; ν_{max} 3190, 3060, 1690 cm^{-1}; λ_{max} 213 (ε 30,800), 246 (7900), 294 (2300), λ_{infl} 240 mμ (7400); NMR: 2·47 (1H, s, —NH), 5·35 (1H, q, J = 7 c/s), 6·05 (3H, s, —OMe), 7·75 (9H, s), 8·53 (3H, d, J = c/s). (Found: C, 71·34; H, 7·94; N, 6·57. $C_{13}H_{17}O_2N$ requires: C, 71·20; H, 7·82; N, 6·39%.) Removal of the solvent from the ether layer left an oil (176 mg) which gave a further amount of XVII (38 mg) on treatment with AcOH (3 ml) and conc HCl (3 ml) by heating under reflux for 8 hr. The water layer was made alkaline with NH_3 and extracted with ether to give an oil (74 mg) which, on recrystallization from ether–pet. ether, afforded prisms, m.p. 101–102°; ν_{max} 3400, 3310, 1735 cm^{-1}, λ_{max} 249·5 (ε 11,000), 297 (3300), λ_{infl} 290·5 mμ (3100). NMR; 6·03 (3H, s, —OMe), 7·55 (3H, s, ArMe), 7·72 (6H, s, 2ArMe), 7·72 (2H, superimposed broad band, $—NH_2$), 8·20 (3H, s, $\underline{Me}C(NH_2)—OCO—$). (Found: C, 66·59; H, 7·43; N, 5·89. $C_{13}H_{17}O_3N$ requires: C, 66·36; H, 7·28; N, 5·95%.)

Treatment of VII *with lead tetraacetate*

Lead tetraacetate (737 mg, 1·65 mmoles) was added under N_2 to a soln of VII (crude, oily in state, 319 mg 1·10 mmoles) in AcOH (6·4 ml), and stirred and maintained at 60°. After 7 hr when the evolution of CO_2 ($Ba(OH)_2$ trap) became sluggish, it was worked up in the usual manner and gave a neutral product (198 mg) which was hydrolyzed with 2N NaOH (7·5 ml) and MeOH (15 ml) by heating under reflux for 1 hr. The ether extracts from the alkaline and the acidified soln weighed 34 mg and 78 mg respectively. These were combined and chromatographed on a silica gel column (3 g). Elution with $CHCl_3$ furnished, after recrystallization from ether–pet. ether, *7-methoxy-3,4,5,6-tetramethyl-1-oxo-1,3-dihydroisobenzofuran* (XIX), m.p. 88–90°; ν_{max} 1745 cm^{-1}; λ_{max} 213 (ε 35,700), 248 (10,200), 298 mμ (3100); NMR: 4·54 (1H, q, J = 7 c/s), 6·02 (3H, s), 7·76 (6H, s), 7·80 (3H, s), 8·40 (d, J = 7 c/s). (Found: C, 70·90; H, 7·54. $C_{13}H_{16}O_3$ requires: C, 70·89; H, 7·32%.)

Potassium permanganate oxidation of VII

$KMnO_4$ soln (1·90 g; 12 mmoles) in water (80 ml) was added portionwise during 40 min to a stirred soln of VII (280 mg, 1 mmole) in 5% NaOH aq (20 ml) at 80°. After stirring 4 hr at this temp, the reaction mixture was added to $NaHSO_3$ (3 g) and 6N H_2SO_4 to decompose the precipitated MnO_2. The product was extracted continuously with ether and methylated with ethereal CH_2N_2. The methylated product was chromatographed over neutral alumina (Act. II, 8·5 g) and elution with benzene gave *methyl 1-(2,4,5-tricarbomethoxy-3-methoxy-6-methylphenyl)propionate* (XX; 63 mg), needles (from ether–pet. ether), m.p. 87–88°; ν_{max} 1720, 1580 cm^{-1}; NMR: 5·50 (3H, s, —OMe), 5·60 (6H, s, 2 $ArCO_2Me$), 5·87 (3H, s, $R—CO_2Me$), 7·39 (3H, s, ArMe), 8·32 (d, J = 7 c/s, $MeCH—$). (Found: C, 56·80; H, 6·09. $C_{18}H_{22}O_9$ requires: C, 56·54; H, 5·80%.) Benzene–ether (4:1) eluted *methyl 1-(2,4,6-tetracarbomethoxy-3-methoxyphenyl)propionate* (XXI; 47 mg), needles (from ether–pet. ether), m.p. 91–92·5°; ν_{max} 1730, 1580, 1565 cm^{-1}. (Found: C, 53·74; H, 5·39. $C_{19}H_{22}O_{11}$ requires: C, 53·52; H, 5·20%.)

Ozonolysis of sclerin

Ozonized O_2 was bubbled through a soln of sclerin (234 mg) in CH_2Cl_2 (15 ml) at $-20°$. After addition of water, the mixture was allowed to stand at room temp for 30 min and then heated on boiling water bath for 20 min. The volatile material formed was introduced into a $2,4\text{-}(NO_2)_2C_6H_3NHNH_2$.HCl trap by means of a N_2 stream. The resulting yellow ppt (35 mg) was recrystallized from EtOH to give yellow crystals, m.p. 173–174°, which were identified as diacetyl mono-2,4-dinitrophenylhydrazone by comparison with an authentic sample (mixed m.p. and IR).[16]

1-(5-*Hydroxy*-2,3,4-*trimethylphenyl*)*propionic acid* (XXII)

Sclerin (400 mg) was heated with KOH (8·0 g) and water (12 ml) in a sealed tube at 180° for 3·5 hr. Acidification followed by ether extraction furnished an oily product (359 mg), which was passed through a column of silica gel (8·0 g). Recrystallization of the $CHCl_3$ eluate from $CHCl_3$–benzene afforded XXII, m.p. 128–130°, ν_{max} 3340, 3240, 2400, 1710, 1600, 865 cm^{-1}; λ_{max} 208·5 (ε 24,200), 290 (2400); λ_{infl} 222 (9400), 284 mμ (2300). (Found: C, 69·20; H, 7·84. $C_{12}H_{16}O_3$ requires: C, 69·21; H, 7·74%.) Methylation of XXII with ethereal CH_2N_2 for 10 min afforded *methyl* 1-(5-*hydroxy*-2,3,4-*trimethylphenyl*)*propionate* (XXV), prisms (from pet. ether), m.p. 114–115°, ν_{max}^{KBr} 3450, 1710, 1590 cm^{-1}. (Found: C, 70·12; H, 8·16. $C_{13}H_{18}O_3$ requires: C, 70·24; H, 8·16%.) XXII (40 mg) was methylated in the usual manner with Me_2SO_4 (0·30 g), anhyd K_2CO_3 (1·5 g) and dry acetone (7·5 ml). Recrystallization of the product from pet. ether gave *methyl* 1-(5-*methoxy*-2,3,4-*trimethylphenyl*)*propionate* (XXIII), prisms, m.p. 42–42·5°; ν_{max} 1735 cm^{-1}; NMR: 3·52 (1H, s, ArH), 6·12 (1H, q, J = 7 c/s), 6·26 (3H, s, —OMe), 6·42 (3H, s, —CO_2Me), 7·83 (6H, s), 7·91 (3H, s), 8·61 (3H, d, J = 7 c/s). (Found: C, 71·23; H, 8·55. $C_{14}H_{20}O_3$ requires: C, 71·16; H, 8·53%.) XXII was refluxed with Ac_2O for 6 hr. The product was passed through a silica gel column and the $CHCl_3$ eluate was recrystallized from ether–pet. ether to give 1-(5-*acetoxy*-2,3,4-*trimethylphenyl*)*propionic acid* (XXVI) as needles, m.p. 110–113°; ν_{max} 3500–2400, 1760, 1692 cm^{-1}. (Found: C, 67·87; H, 7·33. $C_{14}H_{18}O_4$ requires: C, 67·18; H, 7·25%.) XXIII (186 mg) was hydrolyzed with 5% NaOH in MeOH–H_2O (2:1) by heating under reflux for 2 hr. Recrystallization of the product from pet. ether afforded 1-(5-*methoxy*-2,3,4-*trimethylphenyl*)-*propionic acid* (XXX; 107 mg), m.p. 132°; ν_{max} 3400–2200, 1700 cm^{-1}. (Found: C, 70·11; H, 8·22. $C_{13}H_{18}O_3$ requires: C, 70·24; H, 8·16%.)

1-(5-*Methoxy*-2,3,4-*trimethylphenyl*)*ethanol* (XXIV)

XXIII (51 mg) in abs ether (5 ml) was stirred with a suspension of LAH (100 mg) in abs ether (10 ml) for 1 hr at room temp and 1·5 hr by heating under reflux. Addition of 2N HCl and followed by extraction with ether furnished XXIV, plates (from ether–pet. ether), m.p. 52–53°; ν_{max} 3480, 1600, 1295, 1180, 1037, 845 cm^{-1}. (Found: C, 74·90; H, 9·55. $C_{13}H_{20}O_2$ requires: C, 74·96; H, 9·68%.)

Oxidation of XXII *with potassium nitrosodisulphonate (Fremy's salt)*[17]

$ON(SO_3K)_2$ (268 mg) in water (20 ml) was added to a solution of XXII (208 mg, 1 mmole) in 1N NaOH (2 ml). The mixed soln immediately became reddish orange in colour and gradually turned to dark red. Further amounts (2 equivs) of the oxidizing agent was added in two portions within every 10 min. After allowing to stand at room temp for 15 min, the reaction mixture was acidified with 6N H_2SO_4 and extracted with ether. The extract (67 mg) was chromatographed on a silica gel column (3·3 g) and the benzene elute was crystallized from benzene to give XXVII as red rectangular plates; ν_{max} 3200, 2400, 1700, 1660, 1640, 1570 cm^{-1}; λ_{max} 440 (ε 1600), λ_{infl} 263 mμ (2900). (Found: C, 68·72; H, 6·61. $C_{12}H_{14}O_4 \cdot \frac{1}{2}C_6H_6$ requires: C, 68·95; H, 6·56%.)

Tetramethyl-o-benzoquinone

This compound was prepared according to the method of Smith and Hac,[13] and has the following physical properties: m.p. 111° (blacken at 84·5°); ν_{max} 1660, 1623, 1350 cm^{-1}; λ_{max} 448 mμ (ε 1080); $\lambda_{max}^{n\text{-heptane}}$ 423 mμ (ε 1470); NMR, 8·08, 8·25 (each 6H, s).

Potassium permanganate oxidation of XXIII

Compound XXIII (260 mg, 1·11 mmoles) dissolved in 5% NaOHaq (20 ml) was oxidized in the same manner as described for VII with $KMnO_4$ (2·11 g, 13·3 mmoles) in water (20 ml) at 80°. The gummy product (212 mg) was methylated with ethereal CH_2N_2 and then chromatographed over neutral alumina (Act. II, 8·5 g) to afford, on benzene elution, *methyl* 1-(3,4-*dicarbomethoxy*-5-*methoxy*-2-*methylphenyl*)*propionate* (XXIX), needles (from ether–pet. ether), m.p. 64–65·5°; ν_{max} 1722, 1704, 1595, 1580 cm^{-1}; NMR: 3·20 (1H,

s, ArH), 6·13, 6·16 (9H, each s, —OMe and 2-CO_2Me), 6·37 (3H, s, —CO_2Me), 7·83 (3H, s, ArMe), 8·60 (3H, d, J = 7 c/s). (Found: C, 59·45; H, 6·44. $C_{16}H_{20}O_7$ requires: C, 59·25; H, 6·22%.)

Degradation of XXX *to* XXXII*

Compound XXX (83 mg, 0·45 mmoles) in AcOH (2·29 ml) was added to freshly prepared $Pb(OAc)_4$ (254 mg, 0·7 mmole) in portions at 60°. The mixture was stirred for 4 hr at this temp under N_2, $NaHCO_3$ aq was added and the neutral product was extracted with ether to give 1-(5-*methoxy*-2,3,4-*trimethylphenyl*)-*ethyl acetate* (XXXI) as an oil (70 mg), ν_{max}^{film} 1733, 1250 cm^{-1}. XXXI was hydrolyzed with aq methanolic NaOH in the usual manner. Fractionation of the product by crystallization from pet. ether gave rise to an undefined alcohol (8 mg), m.p. 121–124°; ν_{max} 3680, 3620, 3420, 1695, 1685, 1300, 1140, 1075, 980, 905, 870, 855 cm^{-1} and 1-(5-*methoxy*-2,3,4-*trimethylphenyl*)*ethanol* (XXXII) m.p. 61–62°; $\nu_{max}^{CHCl_3}$ 3680, 3400, 3000, 1600, 1470, 1305, 1125, 1042, 1010, 905, 870 cm^{-1}; NMR: 3·19 (1H, s, ArH), 4·96 (1H, q, J = 7 c/s), 6·21 (3H, s, —OMe), 7·86 (3H, s, ArMe), 7·91 (6H, s, ArMe), 8·42 (1H, br. s, —OH), 8·68 (3H, d, J = 7 c/s, MeCH—), identified by the comparison with a synthetic specimen[14] (mixed m.p., TLC, IR, NMR).

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* Experiment by S. Maeda.