THE STRUCTURES OF SCLERIN AND SCLEROLIDE, METABOLITES

OF SCLEROTINIA LIBERTIANA (1)

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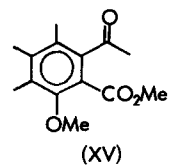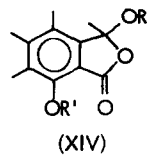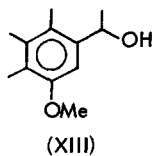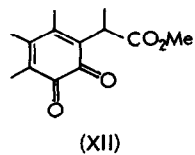
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During the search for the lipase formation stimulating factor in <u>Sclerotinia libertiana</u>, one of the authors (Y.S.) succeeded in isolating the active substance, $C_{13}H_{14}O_4$ (MS m/e 234), m.p. 123°, $[\alpha]_D$ +7.85°, which was designated sclerin (2). Further study on this compound revealed a number of interesting physiological activities — specially the growth promoting effect for various plants. The second minor metabolite, $C_{12}H_{14}O_4$, m.p. 163°, was isolated from the mycelium by a larger scale extraction, and the name sclerolide is proposed for it.

The present paper concerns with the structure elucidations of sclerin and sclerolide. Sclerin exhibited the following spectral properties: $\nu_{max}^{CHCl_3}$ 3260, 1800, 1690, 1610, 1580, 1390, 1345, 1325 cm^{-1}; λ_{max}^{EtOH} 215.5 (ϵ 23100), 263 (7900), 333 mμ (3500); $\lambda_{max}^{0.01N\ KOH-EtOH}$ 313 (4200), λ infl. 246 mμ (6100); n.m.r. −0.80 (1H, s.), 5.86 (1H, q., J=7 cps), 7.83, 7.78, 7.72 (3 methyl singlets), 8.47 τ (methyl doublet, J=7 cps). It gave a greenish-blue color in ferric chloride test, and a mono-acetate (II, R=Ac), m.p. 156°; $\nu_{max}^{CHCl_3}$ 1790, 1760, 1735, 1585, 1195 cm^{-1}; λ_{max}^{EtOH} 216 (ϵ 25500), 260 (8500), 313 mμ (2600) upon acetylation, and hence is a mono-hydric phenol. The hydroxyl group must be in chelation as inferred from the position

of the hydrogen stretching band (3260 cm^{-1}) in the infrared spectrum and hydroxyl

proton signal (-0.80 τ) in the NMR spectrum. The presence of an anhydride grouping

was assumed from the infrared peaks at 1790 and 1735 cm^{-1} in the acetate (II, R=Ac),

and 1785 and 1738 cm^{-1} in the methyl ether (II, R=Me) which was derived from

sclerin through acid hydrolysis of the methyl ether methyl ester acid (III, R=H, R'=R''=

Me), (vide infra) and acetic anhydride treatment of the resultant dicarboxylic acid.

In the sclerin molecule, one of the carbonyl groups in the anhydride structure has to

be responsible for the chelated ring formation mentioned above. On the other hand

the strong absorption in the ultraviolet region indicates the presence of a carbonyl

directly attached to the aromatic ring. With these considerations, the rather wide

separation of anhydride twin bonds (1800 and 1690 cm^{-1}) in the infrared spectrum was

easily understandable and the partial structure (I) was derived. This was extended to

the completed structure (II, R=H) with the aid of NMR data of sclerin, which indicated

the presence of three methyl groups on the aromatic ring and a methyl group linked to

a secondary carbon atom. The calculated value for the major electron transfer band

(3) of sclerin (II, R=H) was 255 mμ, which is in fair agreement with the observed value

of 263 mμ.

Various transformations of sclerin were reasonably interpretable in terms of the

structure (II, R=H) and thus validate the conclusion. Reduction of sclerin with an

excess of sodium borohydride in ethanol solution at room temperature yielded a

lactone (IV, R=H), m.p. $96°$; ν_{max}^{Nujol} $3130, 1665, 1610, 1570$ cm^{-1}; λ_{max}^{EtOH} 217 (ϵ

27700), 260 (10100), 330 mμ (3900); n.m.r. -1.49 (s., chelated -OH), 5.57 (m.,

$-\overset{|}{C}HC\underline{H}_2OCO-$), 6.86 (m., $-C\underline{H}CH_2-$), $7.76, 7.81, 7.85$ (each s., 3 ArCH$_3$), 8.71 τ

(d., J=7.5 cps, $-\overset{|}{C}HC\underline{H}_3$), which was accompanied by a cyclic ether V, m.p. $134°$;

ν_{max}^{Nujol} $3400, 1610, 1585$ cm^{-1}; λ_{max}^{EtOH} 212 (ϵ 13100), 280 mμ (700); n.m.r. 5.26 (s.,

(I)

(II)

(III) CO_2R / CO_2R' / OR''

(IV) OR

(V) OH

(VI) CONH_2 / CO_2Me / OMe

(VII) NR / OMe

(VIII) NH / OMe

(IX) OMe

(X) MeO_2C / MeO_2C / CO_2Me / CO_2Me / OMe / R

(XI) CO_2R / OR'

(XII) CO_2Me

(XIII) OH / OMe

(XIV) OR / OR'

(XV) CO_2Me / OMe
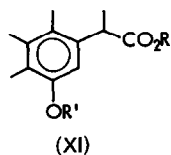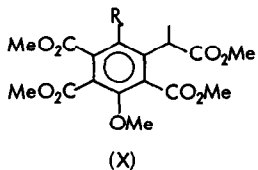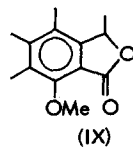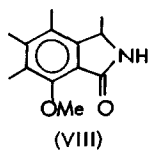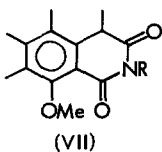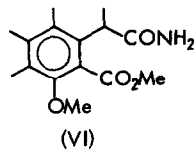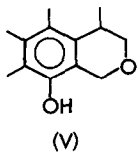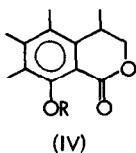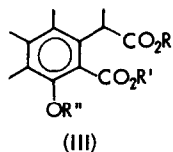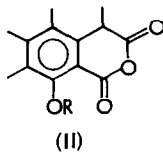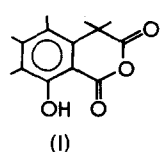
-OH), 4.99, 5.38 (AB quartet, J=16 cps, ArCH$_2$O-), 6.14 (m., -$\overset{|}{\text{C}}$HCH$_2$O-), 7.12 (m.,

-$\overset{|}{\text{C}}$HCH$_2$O-), 7.84 (s., 3 ArCH$_3$), 8.71 τ (d., J=7 cps, CH$_3$$\overset{|}{\text{C}}$H-) on treatment at an

elevated temperature (45°) (4). The lactone (IV, R=H) gave a methyl ether (IV, R=

Me), m.p. 134°; ν$_{max}^{Nujol}$ 1710 cm^{-1}, λ$_{max}^{EtOH}$ 213 (ε 36000), 253 (9100), 305 mμ (2400).

Methylation of sclerin with dimethyl sulfate and potassium carbonate in acetone

solution gave an oily methyl ether dimethyl ester (III, R=R'=R"=Me), ν$_{max}^{CHCl_3}$ 1725 cm^{-1}

which, on hydrolysis with dilute alkali, furnished a methyl ether methyl ester acid (III,

R=H, R'=R"=Me), m.p. 94°; ν_{max}^{Nujol} 1725 cm^{-1}; $\nu_{max}^{CHCl_3-Et_3N}$ 1720, 1590 cm^{-1}; λ_{max}^{EtOH}

211 (ϵ 19400), 280 mμ (1100). The half ester (III, R=H, R'=R"=Me) was converted to an

amide (VI), m.p. 113°; ν_{max}^{Nujol} 3400, 3440, 3300, 3220, 1735, 1720, 1690 cm^{-1}; λ_{max}^{EtOH}

212.5 (ϵ 19900), 283 mμ (2100), which, on acid treatment, yielded lactim (VII, R=H),

m.p. 229°; ν_{max}^{Nujol} 3180, 3060, 1700, 1660, 1615 cm^{-1}; λ_{max}^{EtOH} 210 (ϵ 25300), 270

(8900), 339 (3600), λ infl. 224 mμ (13100). Curtius degradation of III (R=H, R'=R"=Me)

gave a five-membered lactam VIII, m.p. 210°; ν_{max}^{Nujol} 3190, 3060, 1690 cm^{-1}; λ_{max}^{EtOH}

213 (ϵ 30800), 246 (7900), 294 (2300), λ infl. 240 mμ (7400); n.m.r. 2.47 (s., =NH),

5.35 (q., J=7 cps, -$\overset{|}{\text{C}}$HCH$_3$) 6.05 (s., -OCH$_3$), 7.75 (3 ArCH$_3$), 8.53 τ (d., J=7 cps,

-$\overset{|}{\text{C}}$HCH$_3$); reaction of III (R=H, R'=R"=Me) with lead tetracetate in glacial acetic acid

followed by alkaline hydrolysis afforded a five-membered lactone IX, m.p. 90°;

ν_{max}^{Nujol} 1745 cm^{-1}; λ_{max}^{EtOH} 213 (ϵ 35700), 248 (10200), 298 mμ (3100); n.m.r. 4.54 (q.,

J=7 cps, -$\overset{|}{\text{C}}$HCH$_3$), 6.02 (s., -OCH$_3$), 7.76 (s., ArCH$_3$), 7.80 (s., ArCH$_3$), 8.40 τ (d.,

J=7 cps, -$\overset{|}{\text{C}}$HCH$_3$). Upon oxidation with alkaline potassium permanganate at 80°, the

half ester (III, R=H, R'=R"=Me) furnished, after methylation with ethereal diazomethane,

a tetracarboxylic acid methyl ester (X, R=CH$_3$); m.p. 88°; ν_{max}^{Nujol} 1720, 1580 cm^{-1};

n.m.r. 5.50 (s., -OCH$_3$), 5.60 (s., 2 -CO$_2$CH$_3$), 5.87 (s., -CO$_2$CH$_3$), 7.39 (s.,

ArCH$_3$), 8.32 τ (d., J=7 cps, -$\overset{|}{\text{C}}$HCH$_3$) and a pentacarboxylic acid methyl ester (X,

R=CO$_2$CH$_3$), m.p. 92.5°; ν_{max}^{Nujol} 1730, 1580, 1565 cm^{-1}, leaving the 1-carboxyethyl

side chain intact. The most hindered ring-methyl presumably remained unattacked in

the former. When sclerin was heated with 40% aq. potassium hydroxide solution in a

sealed tube at 180°, decarboxylation occurred to yield a nor-acid (XI, R=R'=H), m.p.

126°; ν_{max}^{Nujol} 3700, 3300, 2450, 1705, 1600, 860 cm^{-1}; λ_{max}^{EtOH} 208.5 (ϵ 24200), 290

(2400), λ infl. 222 (9400), 284 mμ (2300); methyl ether methyl ester (XI, R=R'=Me),

m.p. 42.5°, ν_{max}^{Nujol} 1735 cm^{-1}; n.m.r. 3.52 (s., Ar-H), 6.12 (q., J=7 cps, -$\overset{|}{\text{C}}$HCH$_3$),

6.26, 6.42 (each s., $-OCH_3$ and $-CO_2CH_3$), 7.83 (s., 2 $ArCH_3$), 7.91 (s., $ArCH_3$),

8.61 τ (d., J=7 cps, $-\overset{|}{C}HC\underline{H}_3$). Oxidation of the nor-acid (XI, R=R'=H) with potass-

ium nitrosodisulphonate yielded an o-quinone (XII), ν_{max}^{Nujol} 3200, 2400, 1700, 1660,

1640, 1570 cm^{-1}, λ_{max}^{EtOH} 440 (ϵ 1600), λ infl. 263 mμ (2900), the physical data of

which showed close resemblance to those of tetramethyl-o-benzoquinone, ν_{max}^{Nujol} 1660,

1623, 1350 cm^{-1}; λ_{max}^{EtOH} 447 mμ (ϵ 1140) (5). The methyl ether acid (XI, R=H, R'=Me),

obtainable from XI (R=R'=Me) was degraded, through lead tetracetate oxidation and

subsequent hydrolysis, to an alcohol (XIII), m.p. 62°; ν_{max}^{Nujol} 3840, 3460, 1600 cm^{-1},

which was identical with a synthetic specimen (IR). Eventually the nor-acid (XI, R=

R'=H) was also synthesized by way of XIII and the identity with the compound derived

from sclerin was confirmed (IR). The substitution pattern on the benzene nucleous is

thus fully secured and the structure assignment of sclerin as II (R=H) is proved con-

clusively.

The second metabolite, sclerolide (XIV, R=R'=H), m.p. 163°; ν_{max}^{Nujol} 3420, 1720

cm^{-1}; λ_{max}^{EtOH} 213 (ϵ 16300), 250 (7700), 313 mμ (4300); n.m.r. 2.31 (s., -OH), 7.69,

7.76, 7.82 (each s., 3 $ArCH_3$), 8.11 τ (s., $\geqslant CCH_3$) lacks a carbon atom and has an

additional hydroxyl group as compared to sclerin. Thus it gave a blue color in ferric

chloride test and yielded a diacetate (XIV, R=R'=Ac), m.p. 163°; ν_{max}^{Nujol} 1780, 1760,

1605 cm^{-1}; λ_{max}^{EtOH} 211.5 (ϵ 47000), 250.0 (11000), 296 (2600), λ infl. 290 mμ (2500);

n.m.r. 7.60 (s., $ArOCOCH_3$), 7.72, 7.74, 7.85 (each s., $ArCH_3$), 8.01 (s. $ROCOCH_3$),

8.09 τ (s., $\geqslant CCH_3$). The position of carbonyl stretching bands of sclerolide (XIV, R=

R'=H) and its diacetate (XIV, R=R'=Ac) in the infrared spectra (1720 and 1780 cm^{-1}

respectively) indicated the presence of five-membered lactonic functions in these com-

pounds and that the lactone carbonyl in the former was intramolecularly hydrogen-

bonded. There is a close resemblance in the ultraviolet spectrum of sclerolide to that

of sclerin, which suggests the existence of similar chromophores in both compounds. On the other hand the only marked difference in their NMR spectra is that sclerolide exhibits a singlet, due to the methyl group linked to tertiary carbon which in turn bears a strongly negative substituent, in place of A_3X pattern signals in the case of sclerin. These considerations lead to expression XIV (R=R'=H) as the only plausible structure for sclerolide. Methylation of sclerolide with ethereal diazomethane furnished a dimethyl ether methyl ester (XV, R=R'=Me), m.p. 57°; ν_{max}^{Nujol} 1720, 1695 cm^{-1}; λ_{max}^{EtOH} 213 (ϵ 19800), 291 (1700), λ infl. 245 mμ (6300); n.m.r. 6.16 (s., -OCH$_3$), 6.26 (s., -CO$_2$CH$_3$), 7.52 (s., ArCOCH$_3$), 7.79, 7.77, 7.84 τ (each s., 3 ArCH$_3$) in which the presence of a nuclear acetyl group is revealed from the spectral evidences cited. Upon alkaline hydrolysis and subsequent acidification it yielded an alcohol (XIV, R=H, R'=Me), m.p. 144°; ν_{max}^{Nujol} 3370, 1750 cm^{-1}; λ_{max}^{EtOH} 213 (ϵ 34600), 250 (9500), 300 mμ (2700); n.m.r. 5.27 (s., -OH), 6.14 (s., -OCH$_3$), 7.63, 7.68, 7.84 (each s., 3 ArCH$_3$), 8.16 τ (s., $\geqslant$C-CH$_3$). These transformations provide strong support for the presence of the lactol grouping in sclerolide. Final proof for the structure XIV (R=R'=H) comes from a chemical correlation with sclerin. The treatment of sclerolide (XIV, R=R'=H) with sodium amalgam at room temperature afforded crystals of m.p. 103°, which was identified as IX through the comparison of infrared spectra.

REFERENCES

1. A preliminary report of this work was presented at the 8th Symposium on the Chemistry of Natural Products (Japan), Nagoya, Oct. 20th, 1964.

2. Y. Satomura and A. Sato, Agr. Biol. Chem. (Japan) 29, 337 (1965).

3. A. I. Scott, Interpretation of the Ultraviolet Spectra of Natural Products, p. 89-134. Pergamon Press, Oxford (1964).

4. cf. W. R. Vaughan, C. T. Goetschel, M. H. Goodrow and C. L. Warren, J. Am. Chem. Soc. 85, 2282 (1963).

5. L. I. Smith and L. R. Hac, J. Am. Chem. Soc. 56, 477 (1934).